Table S1, related to Figures 2, 3, 4, S3, S4, S5 and Table 1.

A. *sfr1Δ*, *rhp55Δ*, and *rhp57Δ* Mutations Partially Suppress The Low Viable Spore Yield of *mus81Δ* Strains

Mutant	Viable spores/cell[1]	
	mus81^{+}	*mus81Δ*
wild type	≡ 100 (16)	0.005 ± 0.001 (12)[2]
swi5Δ	33 ± 7.5 (4)	–
sfr1Δ	50 ± 5.8 (8)	0.03 ± 0.01 (4)[2]
swi5Δ sfr1Δ	43 ± 16 (4)	–
rhp55Δ[3]	1.2 ± 0.6 (4) 11.7 ± 2.4 (4)	0.6 ± 0.03 (8)
rhp57Δ[3]	2.4 ± 1.6 (4) 8.4 ± 4.0 (4)	0.17 ± 0.03 (8)

[1]Mating and meiosis were on SPA at 25°. Viable spore yields are expressed relative to wild-type matings, which produced 3.8 ± 0.52 viable spores per viable cell (of the less numerous parent) added to the mating mixture. Data are the mean ± SEM (number of matings in parentheses).

[2] The relative viable spore yield for *mus81Δ* was 0.001 ± 0.0006 (3) on the same day the yield for *sfr1Δ mus81Δ* was 0.03 ± .01 (4), for a 30-fold increase.

[3] Viable spore yields for the *rhp55Δ* and *rhp57Δ* strains were performed on two separate days, and two values are given due to significant difference in the measurement from each day. All measurements performed on different days (*i.e.,* n > 4) showed no significant difference unless noted.

B. Dmc1- and Swi5-dependence of Intergenic Recombination

Intergenic interval tested	Fraction recombinants (%; **cM**)[1] [number of crosses]		
	dmc1$^+$ *swi5*$^+$	*dmc1Δ*	*swi5Δ*
lys3 – aur1	206/1816 (11.1; **12.5**) [5]	23/1206 (1.9; **1.9**) [2]	3/954 (0.3; **0.3**) [2]
lys3 – ura1	299/1548 (19.3; **24.4**) [4]	—[2]	—
lys3 – ura1 (*mbs1Δ*)[3]	501/2530 (19.8; **25.2**) [6]	51/1226 (4.2; **4.3**) [2]	24/954 (2.5; **2.6**) [2]
ura1 – rqh1	262/1772 (14.7; **17.4**) [5]	22/715 (2.9; **3.0**) [1]	—
ura1 – rqh1 (mbs1Δ)	250/2530 (9.9; **11.0**) [6]	12/1226 (1.0; **1.0**) [2]	5/954 (0.5; **0.5**) [2]
ura2 – leu2	22/1050 (2.1; **2.1**) [4]	5/910 (0.55; **0.55**) [2]	1/210 (0.5; **0.5**) [1]
ura2 – leu2 [4]	3.2%, 3.4% (**3.4**) [2]	1.1%, 0.8% (**1.0**) [2]	0.2%, 0.3% (**0.3**) [2]

[1] Data are the fraction of recombinants among the indicated number of spore colonies tested from the number of crosses in square brackets. The frequencies of reciprocal recombinant types were not significantly different.

[2] Not determined.

[3] *mbs1-19*, a 12 kb deletion.

[4] Data are twice the frequency of Ura$^+$ Leu$^+$ recombinants determined by differential plating on minimal media; >150 colonies of recombinant and parental types were counted in each case.

Supplemental Experimental Procedures

***S. pombe* Strains**

Strain	Genotype
GP13	*h⁻ ade6-52*
GP14	*h⁺ ade6-52*
GP23	*h⁻ ade6-M26*
GP24	*h⁺ ade6-M26*
GP76	*h⁺ura2-10*
GP98	*h⁻ ade6-M26 ura2-10*
GP742	*h⁺lys7-2*
GP786	*h⁺ ade6-M26 lys3-37*
GP3062	*h⁺ ura4-D18 rad50S pat1-114*
GP3198	*h⁻ aur1-18* (Ts)
GP3797	*h⁺ ade6-M26 mus81::kanMX6*
GP3999	*h⁻ ade6-52 swi5-201::kanMX6*
GP4020	*h⁺ ade6-M26 swi5-201::kanMX6*
GP4126	*h⁺ ade6-3049 ura4-D18 mbs1-1::ura4⁺ pat1-114 rad50S end1-458*
GP4262	*h⁺ ade6-3049 mbs1-19 pat1-114 rad50S end1-458*
GP4298	*h⁻ ade6-3049*
GP4381	*h⁻ ade6-3049 mbs1-19 rqh1-h2*
GP4382	*h⁺ ade6-3049 mbs1-19 ura1-61*
GP4383	*h⁻ ade6-3049 mbs1-19 lys3-37*
GP4425	*h⁺ ade6-3049 mbs1-19 rqh1-h2*
GP4428	*h- ade6-3049 mbs1-19 ura1-61 rqh1-h2*
GP4429	*h⁺ ade6-3049 mbs1-19 lys3-37*
GP4431	*h⁻ ade6-3049 mbs1-19 lys3-37 ura1-61*
GP4489	*h⁺ ade6-3049 mbs1-19 lys3-37 ura1-61 rqh1-h2*
GP4490	*h⁻ ade6-3049 mbs1-19 lys3-37 ura1-61 rqh1-h2*
GP4552	*h⁺ ade6-3049 lys3-37 ura1-61 rqh1-h2*
GP4555	*h⁻ ade6-M26 mbs1-19*
GP4556	*h⁺ ade6-M26 mbs1-19*
GP4644	*h⁻ ade6-52 mus81::kanMX6*
GP4646	*h⁻ˢᵐᵗ⁰ ade6-52 ura4-D18 rhp55::ura4⁺*
GP4705	*h⁺ ade6-M26 ura4-D18 rhp55::ura4⁺*
GP4711	*h⁺ ade6-M26 ura4-D18 dmc1::ura4⁺*
GP4712	*h⁻ˢᵐᵗ⁰ ade6-52 ura4-D18 rhp57::ura4⁺*

GP4739 *h^{+} ade6-M26 ura4-D18 rhp57::ura4^{+}*

GP4870 *h^{-smt0} ade6-52 ura4-D18 rhp55::ura4^{+} mus81::kanMX6*

GP5086 *h^{-} ade6-210 mbs1-25 pat1-114 ura1-61*
h^{-} ade6-216 mbs1-24 pat1-114

GP5061 *h^{-} ade6-M26 lys3-37 aur1-18* (Ts)

GP5170 *h^{+} ade6-M26 ura4-D18 sfr1::ura4^{+}*

GP5232 *h^{+} ade6-M375 leu1-32 ura4-D18 dmc1::ura4^{+} rec12-152::LEU2*

GP5247 *h^{-} ade6-3049 leu1-32 ura4-D18 dmc1::ura4^{+}*

GP5324 *h^{-} ade6-3049 sfr1::ura4^{+} ura4-D18*

GP5325 *h+ ade6-3049 swi5-201::kanMX6*

GP5326 *h^{-} ade6-3049 swi5-201::kanMX6*

GP5330 *h^{-} ade6-3057 leu1-32 ura4-D18 dmc1::ura4^{+}*

GP5441 *h^{-smt0} ade6-M26 ura4-D18 mus81::kan MX6*

GP5588 *h^{+} ade6-52 ura4-D18 rhp57::ura4^{+} mus81::kanMX6*

GP5589 *h^{-smt0} ade6-M26 ura4-D18 rhp57::ura4^{+} mus81::kanMX6*

GP5590 *h^{+} ade6-M26 ura5-D18 rhp55::ura4^{+} mus81::kanMX6*

GP5648 *h^{-smt0} ade6-52 ura4-D18 sfr1::ura4^{+}*

GP5678 *h^{+} ade6-M26 ura4-D18 sfr1::ura4^{+} mus81::kanMX6*

GP5682 *h^{-smt0} ade6-52 ura4-D18 sfr1::ura4^{+} mus81::kanMX6*

GP5701 *h^{+} ade6-M26 ura4-D18 sfr1::ura4^{+} swi5::kanMX6*

GP5704 *h^{-smt0} ade6-52 ura4-D18 sfr1::ura4^{+} swi5::kanMX6*

GP5931 *h^{-} ade6-210 mbs1-25 pat1-114 ura4-D18 dmc1::ura4^{+} ura1-61*
h^{-} ade6-216 mbs1-24 pat1-114 ura4-D18 dmc1::ura4^{+}

GP6052 *h^{-} ade6-210 mbs1-25 pat1-114 swi5-201::kanMX6 ura1-61*
h^{-} ade6-216 mbs1-24 pat1-114 swi5-201::kanMX6

GP6104 *h^{+} ade6-3095::ura4^{+} ura4-D18*

GP6371 *h^{+} ade6-210 mbs1-25 pat1-114 his3-D1 rad51::his3^{+} ura1-61*
h^{+} ade6-216 mbs1-24 pat1-114 his3-D1 rad51::his3^{+} lys3-37

GP6372 *h^{-} ade6-210 mbs1-25 pat1-114 ura4-D18 sfr1::ura4^{+} ura1-61*
h^{-} ade6-216 mbs1-24 pat1-114 ura4-D18 sfr1::ura4^{+} lys3-37

GP6384 *h^{+} ade6-210 mbs1-25 pat1-114 swi5-201::kanMX6 mus81::kanMX6 his4-239 ura1-61*
h^{+} ade6-216 mbs1-24 pat1-114 swi5-201::kanMX6 mus81::kanMX6 lys4-95

GP6454 *h^{-} ade6-52 mbs1-103 ura4-D18 dmc1::ura4^{+}*

GP6458 *h^{+} ade6-M26 ura4-D18 dmc1::ura4^{+} ura1-61 rqh1-h2*

GP6464 *h^{-} ade6-52 mbs1-103*

GP6468 *h^{+} ade6-M26 ura1-61 rqh1-h2*

GP6490 *h^{+} ade6-210 mbs1-25 pat1-114 ura4-D18 rhp57::ura4^{+} ura1-61*
h^{+} ade6-216 mbs1-24 pat1-114 ura4-D18 rhp57::ura4^{+}

GP6491 *h^{+} ade6-210 mbs1-25 pat1-114 ura4-D18 rhp57::ura4^{+} mus81::kanMX6 his4-239 ura1-61*

h$^+$ *ade6-216 mbs1-24 pat1-114 ura4-D18 rhp57::ura4*$^+$ *mus81::kanMX6 lys4-95*

GP6591 *h*$^+$ *ade6-3074 ura4-D18 dmc1::ura4*$^+$

GP6603 *h*$^+$ *ade6-3074*

GP6604 *h*$^-$ *ade6-52 ura4-D18 dmc1::ura4*$^+$

GP6605 *h- ade6-210 pat1-114 mbs1-25 ura1-61 ura4-D18 dmc1::ura4*$^+$ *mus81::kanMX6*
h- ade6-210 pat1-114 mbs1-25 lys3-37 ura4-D18 dmc1::ura4$^+$ *mus81::kanMX6*

GP6656 *h*$^-$ *ade6-3049 pat1-114 mbs1-24 bub1-243 lys3-37 his4-239*
h$^-$ *ade6-3049 pat1-114 mbs1-25 vtc4-1104 ura1-61 lys4-95*

GP6657 *h*$^-$ *ade6-3049 pat1-114 mbs1-24 mus81::kanMX6 bub1-243 lys3-37 his4-239*
h$^-$ *ade6-3049 pat1-114 mbs1-25 mus81::kanMX6 vtc4-1104 ura1-61 lys4-95*

GP6658 *h*$^+$ *ade6-210 mbs1-25 pat1-114 ura4-D18 rhp57::ura4*$^+$ *swi5-201::kanMX6 ura1-61*
h$^+$ *ade6-216 mbs1-24 pat1-114 ura4-D18 rhp57::ura4*$^+$ *swi5-201::kanMX6 lys3-37*

GP6715 *h*$^-$ *ade6-3049 pat1-114 mbs1-24 swi5-201::kanMX6 bub1-243 lys3-37 his4-239*
h$^-$ *ade6-3049 pat1-114 mbs1-25 swi5-201::kanMX6 vtc4-1104 ura1-61 lys4-95*

GP6716 *h*$^-$ *ade6-3049 pat1-114 mbs1-24 ura4-D18 dmc1::ura4*$^+$ *bub1-243 lys3-37 his4-239*
h$^-$ *ade6-3049 pat1-114 mbs1-25 ura4-D18 dmc1::ura4*$^+$ *vtc4-1104 ura1-61 lys4-95*

GP6939 *h*$^+$ *ade6-3049 ura4-D18 dmc1::ura4*$^+$

GP6943 *h*$^-$ *ade6-3049 mbs1-19 lys3-37 ura1-61 rqh1-h2 ura4-D18 dmc1::ura4*$^+$

GP6944 *h*$^+$ *ade6-M26 mbs1-19 ura4-D18 dmc1::ura4*$^+$

GP6946 *h*$^+$ *ade6-M26 mbs1-19 swi5-201::kanMX6*

GP6947 *h*$^-$ *ade6-M26 lys3-37 aur1-18* (Ts) *ura4-D18 dmc1::ura4*$^+$

GP6949 *h*$^-$ *ade6-M26 lys3-37 aur1-18* (Ts) *swi5-201::kanMX6*

GP6953 *h*$^-$ *ade6-3049 mbs1-19 lys3-37 ura1-61 rqh1-h2 swi5-201::kanMX6*

GP7044 *h*$^+$ *ade6-3049 leu2-120 lys7-2 dmc1::ura4*$^+$ *ura4-D18*

GP7047 *h*$^-$ *ade6-M26 ura2-10 dmc1::ura4*$^+$ *ura4-D18*

GP7048 *h*$^-$ *ade6-3049 leu2-120 lys7-2 swi5-201::kanMX6*

GP7049 *h*$^-$ *ade6-M26 ura2-10 swi5-201::kanMX6*

GP7051 *h*$^-$ *ade6-3049 leu2-120 lys7-2*

GP7082 *h*$^-$ *ura2-10 leu2-120*

GP7085 *h*$^-$ *leu2-120 lys7-2*

Mutations other than mating type and commonly used auxotrophies are described in the following references: *ade6-M26* (Gutz, 1971; Szankasi et al., 1988), *ade6-3049* and *ade6-3074* (Steiner and Smith, 2005), *swi5-201::kanMX6* (Ellermeier et al., 2004), *dmc1::ura4*$^+$ (Fukushima et al., 2000), *sfr1::ura4*$^+$ (Akamatsu et al., 2003), *rhp55::ura4*$^+$ (Khasanov et al., 1999), *rhp57::ura4*$^+$ (Tsutsui et al., 2000), *rad51::his3*$^+$ (Grishchuk et al., 2004), *rad50S* (Farah et al., 2002), *rqh1-h2* (Stewart et al., 1997), *aur1-18* (Ts) (Hashida-Okado et al., 1998), *mus81::kanMX6* (Boddy et al., 2001), *rec12-*

152::LEU2 (Lin and Smith, 1994), *end1-458* (Uemura and Yanagida, 1984), *pat1-114* (Iino and Yamamoto, 1985), *mbs1-1::ura4*$^{+}$, *mbs1-24*, *mbs1-25,* and *mbs1-103* (Cromie et al., 2005), *mbs1-19*, *bub1-243* and *vtc4-1104* (Supplemental Experimental Procedures). Diploid strains, whose parental genotypes are separated by a line, were constructed by protoplast fusion of parental haploids, essentially as described by Sipiczki and Ferenczy (1977). By automated nucleotide sequence analysis of appropriate PCR products we determined that *ura2-10* changes codon 241 from TCT to ATT (S241I) and *leu2-120* changes codon 581 from GAA to AAA (E581K).

Introduction of Heterozygous Restriction Site Markers

Heterozygous restriction sites to the left (*L*) and right (*R*) of *mbs1* are described in Cromie et al. (2005). At *ade6*, the *L* marker inactivated a *Sca*I site, and the *R* marker inactivated a *Pml*I site. Marker *L* is a TAC → TAT mutation at bp 30617 of cosmid SPCC1322 (GenBank accession no. AL035259.1) [silent mutation at bp 243 (codon 81) of *bub1* ORF], and marker *R* is a GCA → GCG mutation at position 34414 of cosmid SPCC1322 [silent mutation at bp 1104 (codon 368) of *vtc4* ORF]. These changes were made by transforming strain GP6104 with linear DNA carrying the appropriate point mutations (introduced by PCR primer mutagenesis of chromosomal DNA) at the *L* site to create *bub1-243* or the *R* site to create *vtc4-1104*. The marker *ade6-3095* in strain GP6104 is a replacement of bp 30616-34415 of cosmid SPCC1322 with the 1.8-kb *Hin*dIII fragment containing *ura4*$^{+}$*.*

Construction of *mbs1-19* Deletion

The allele *mbs1-19* is an 11.8 kb deletion of bp 2,986 – 14,808 of cosmid c4G8 on chromosome I (GenBank accession no. Z56276.2). Two PCR products with overlapping homology and homology to the left and right deletion end-points were synthesized with *S. pombe* genomic DNA as template. For the first PCR (left deletion endpoint) oligonucleotides 1 (5'-ggcgtagttaatgcaatcagc-3') and 2 (5'-ccgctaagtttaacgaagaaatagccgctgacttcctcaacgtcc-3') were used as primers. For the second PCR (right deletion endpoint) oligonucleotides 3 (5'-ggacgttgaggaagtcagcggctatttcttcgttaaacttagcgg-3') and 4 (5'-ggtgatcttcaacattgatcc-3') were used as primers. These PCR products, 767 and 528 bp respectively, shared 45 bp of homology, as oligonucleotides 2 and 3 are complementary and contain sequences from each side of the deletion point. The PCR products were spin column-purified (Qiagen), combined, and annealed to form a template for a subsequent PCR primed with "outside" oligonucleotides 1 and 4. This 1295 bp PCR product was purified and used to transform strain

GP4126 (*mbs1-1::ura4*$^{+}$ *ura4-D18*) to FOA-resistance. The *mbs1-19* deletion was confirmed by nucleotide sequence analysis.

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