

Supplemental Data

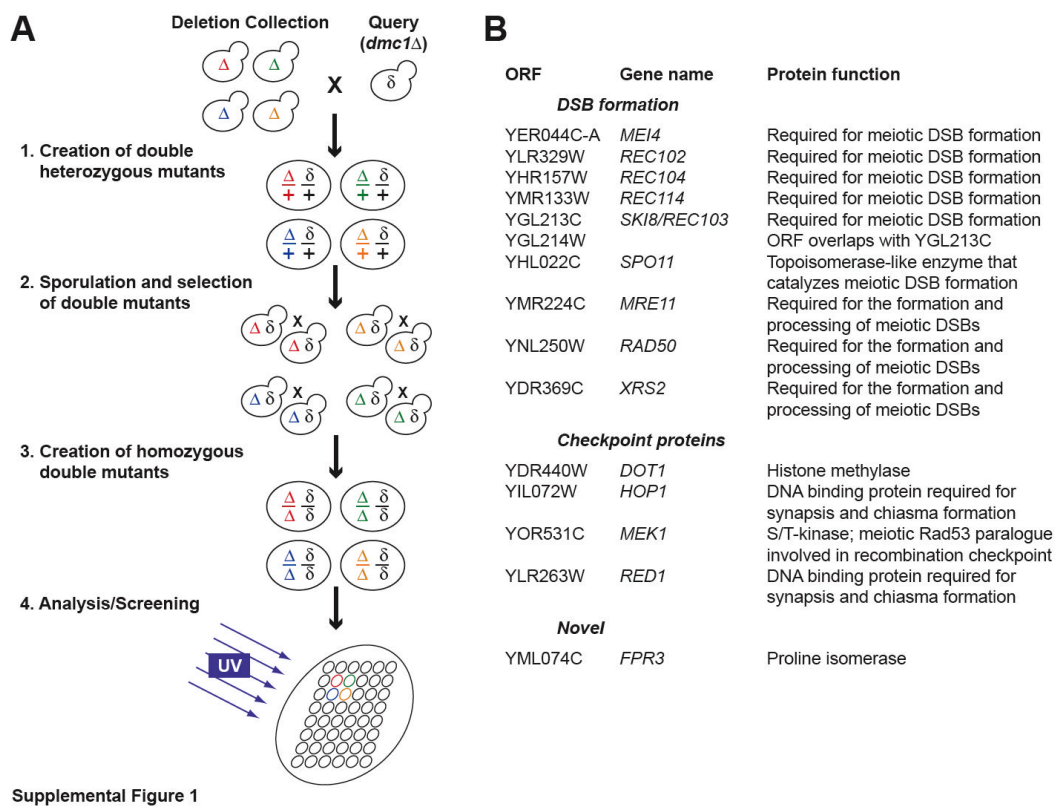
The FK506 Binding Protein Fpr3 Counteracts

Protein Phosphatase 1 to Maintain

Meiotic Recombination Checkpoint Activity

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Supplemental Figures



**Figure S1: A screen to identify components of the recombination checkpoint**

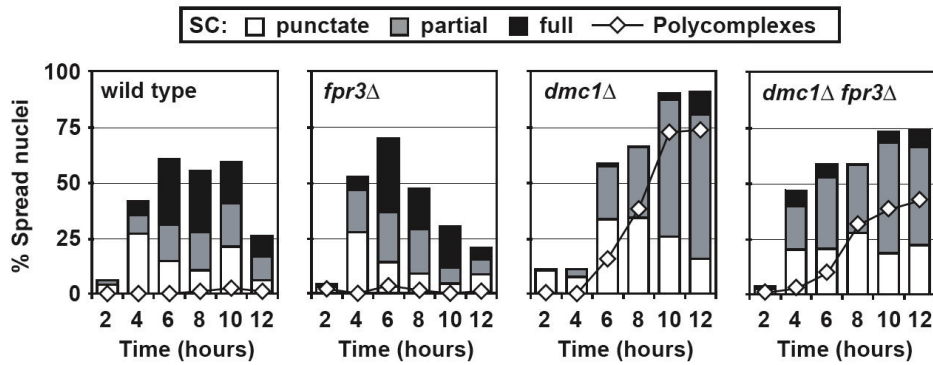
(A) Mature budding yeast spores accumulate dihydroxyacetone in their spore wall, a compound that renders sporulating colonies fluorescent under ultra-violet light (Briza et al., 1986).

We used this assay to screen the *S. cerevisiae* deletion collection (Giaever et al., 2002) for deletions that allowed *dmc1D* mutants to progress through meiosis and form spores.

Outline of the screen:

$\Delta$  denotes a strain from the yeast deletion collection (different colors indicate different mutations),  $\delta$  denotes the query mutation (*dmc1D*). In addition to the query mutation, the query strain (A5054) harbored the *pSTE5-URA3* construct that allowed respective selection for haploid (on medium lacking uracil) and diploid cells (on medium containing 5-fluoro orotic acid) at different steps of the mating scheme. The query mutation, a deletion of the entire ORF of *DMC1* (*dmc1D::HIS3*), was introduced into the deletion collection by mass mating on plates and was subsequently homozygosed as described (Marston et al., 2004). Homozygous double mutants were sporulated for 3 days on a nitrocellulose filter that was placed onto a sporulation plate. The filter served to block out the agar auto-fluorescence and allowed screening by hand-held UV source directly on the plate. A caveat with our screening approach is the fact that a fraction of the deletion collection is known to be aneuploid (Hughes et al., 2000). Indeed, many initially UV-positive candidates were discovered to still harbor a wild-type copy of *DMC1* in addition to the *dmc1D::HIS3* query allele. We therefore isolated DNA of UV-positive mutants and performed a secondary screen, screening by PCR for the absence of a wild-type *DMC1* gene. Only isolates that did not show a wild-type size *DMC1* PCR fragment in this screen were kept for further analysis.

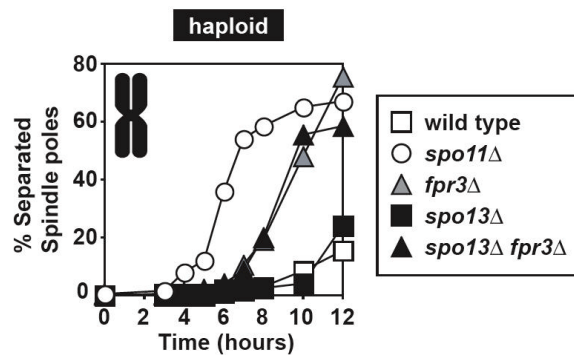
(B) Summary of genes identified in the screen.



Hochwagen et al., Supplemental Figure 2

### Figure S2: The defects in SC formation in *dmc1D* mutants are not rescued by inactivating *FPR3*

Nuclei of wild-type (A7589), *dmc1D* (A7590), *fpr3D* (A7588), and *dmc1D fpr3D* (A7587) cells, all carrying a *ZIP1-GFP* fusion, were spread at the indicated time points, and SC structures were analyzed.

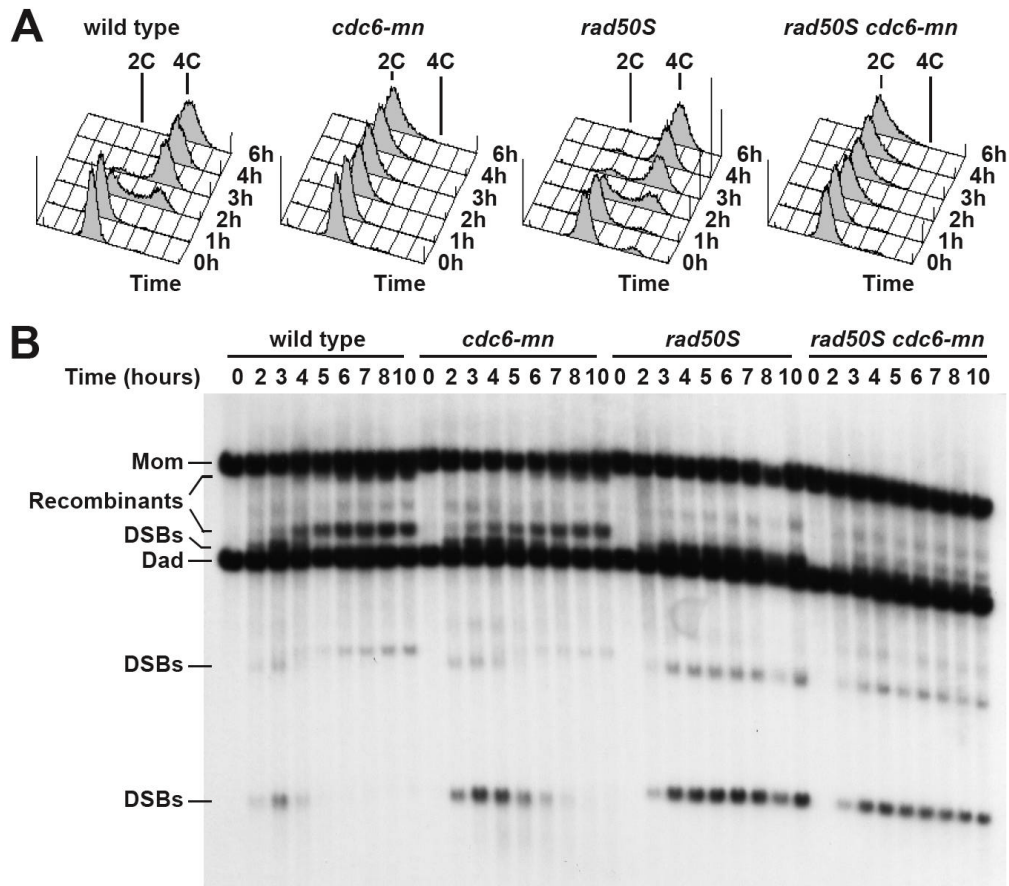


Hochwagen et al., Supplemental Figure 3

### Figure S3: Deletion of *SPO13* does not restore spore viability to *dmc1D fpr3D* mutants

Cells were induced to undergo meiosis and samples were taken at the indicated times to analyze the percentage of cells with separated SPBs. The following strains were used:

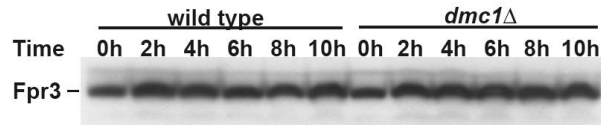
wild type (A8873), *spo11D* (A10272), *fpr3D* (A11288), *spo13D* (A9802), *spo13D fpr3D* (A9799).



Hochwagen et al., Supplemental Figure 4

**Figure S4: *cdc6-mn* mutants form DSBs and undergo recombination without pre-meiotic DNA replication**

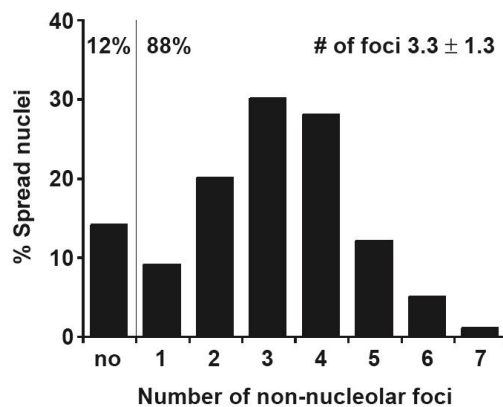
Wild type (NKY1551), *cdc6-mn* (A10912), *rad50S* (A11675), and *rad50S cdc6-mn* (A11265) cells were induced to undergo meiosis and samples were taken at the indicated times to analyze DNA content by flow cytometry (A), and recombination using the *HIS4LEU2* hotspot (Storlazzi et al., 1995) by Southern blot analysis (B). The Southern blot was probed with probe A..



Hochwagen et al., Supplemental Figure 5

### Figure S5: Fpr3 protein levels are constant throughout meiotic development

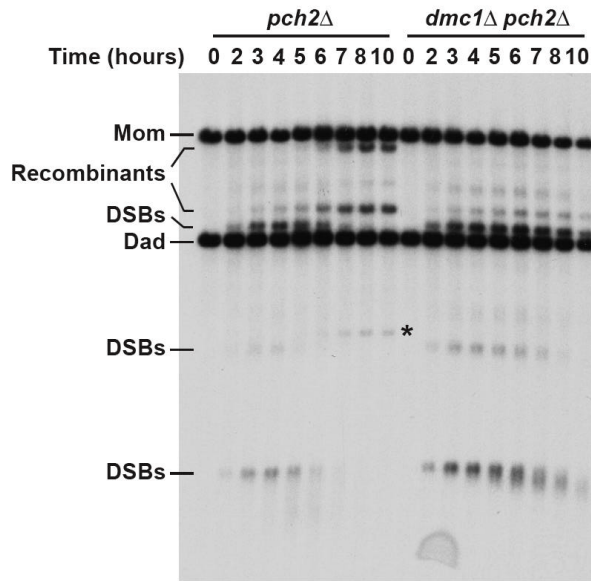
Wild-type (A6871) and *dmc1Δ* (A6872) cells were induced to undergo meiosis and the amount of Fpr3 protein was analyzed Western blot analysis at the indicated times.



Hochwagen et al., Supplemental Figure 6

### Figure S6: Distribution of non-nucleolar chromatin-associated Fpr3 foci present during meiosis

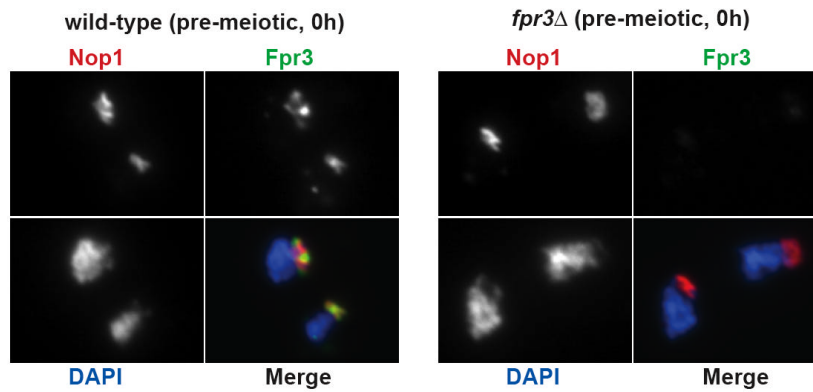
Quantification of non-nucleolar Fpr3 foci from late G1 cells (haploid wild-type (A10416); 30 minutes after release from  $\alpha$ -factor). Percentages on top indicate the number of cells with no extra foci (12%) versus cells with extra foci (88%). Cells with extra Fpr3 foci contained on average  $3.3 \pm 1.3$  (s.d.) foci.



Hochwagen et al., Supplemental Figure 7

**Figure S7: *PCH2* is required for DSB repair**

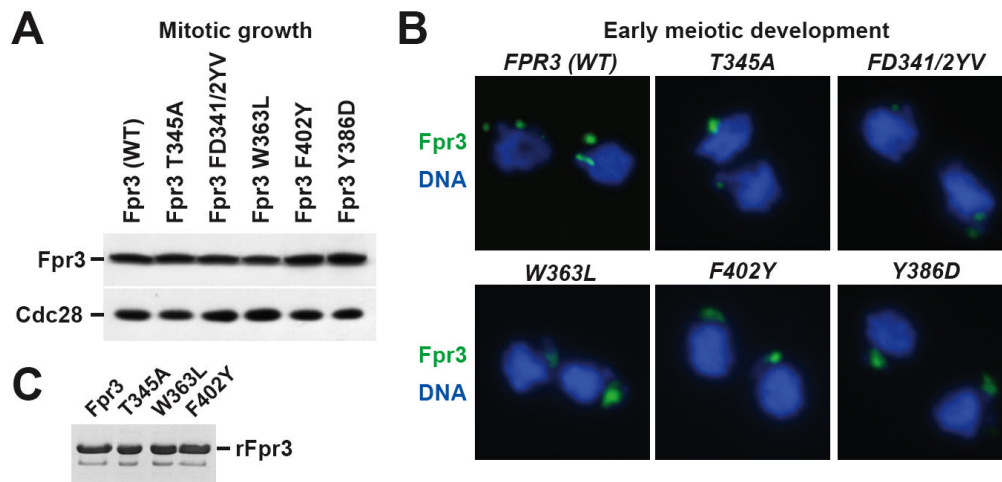
*pch2D* (A11026) and *dmc1D pch2D* (A10843) cells were induced to undergo meiosis and samples were taken at the indicated times to analyze recombination using the *HIS4LEU2* hotspot (Storlazzi et al., 1995) by Southern blot analysis. The Southern blot was probed with probe A. \* indicates a *RAD52*-dependent DNA fragment that is likely to be the result of ectopic recombination of the *HIS4LEU2* locus with *leu2::hisG* locus.



Hochwagen et al., Supplemental Figure 8

### Figure S8: Nop1 localization is not affected by inactivation of *FPR3*

Nop1 localization was analyzed on spread meiotic nuclei of wild-type (A12444), *fpr3D* (A12442).



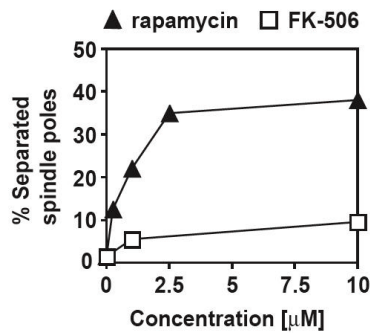
Hochwagen et al., Supplemental Figure 9

### Figure S9: Effects of point mutations in the proline isomerase domain of Fpr3 on protein levels and protein localization during vegetative growth and meiotic development

(A) Protein levels of Fpr3 point mutants in cycling cells were determined by Western blot. Cdc28 serves as loading control. Wild type *FPR3* (A9674), *T345A* (A9675), *F341Y/D342V* (A9676), *W363L* (A9677), *F402Y* (A9678), *Y386D* (A9679).

(B) Localization of Fpr3 point mutants on spread nuclei of wild type *FPR3* (A12658), *T345A* (A12659), *F341Y/D342V* (A12660), *W363L* (A12661), *F402Y* (A12662), *Y386D* (A12663) at the time of transfer into sporulation medium.

(C) 1 $\mu$ g of each purified recombinant Fpr3 protein (wild-type and point mutants) as used in the in vitro PPIase assay.



Hochwagen et al., Supplemental Figure 10

**Figure S10: Effects of various concentrations of rapamycin and FK506 on the recombination checkpoint arrest**

*dmc1 $\Delta$*  (A6872) cells were treated with 1% methanol or different concentrations of rapamycin or FK506 two hours after meiotic induction. The percentage of separated spindle poles 14 hours after meiotic induction was quantified.



## Supplemental Tables

**Table S1: Spore viabilities**

Strain	Genotype	Ploidy	Spore viability	N(spores) <sup>2</sup>
A6871	wild type	diploid	97.5 %	160
A6872	<i>dmc1D</i>	diploid	8 %	40
A8596	<i>fpr3D</i>	diploid	99 %	160
A6683	<i>dmc1D fpr3D</i>	diploid	10 %	40
A9035	<i>spo13D</i>	diploid	46 %	200
A9033	<i>spo13D dmc1D</i>	diploid	17 %	200
A9034	<i>spo13D fpr3D</i>	diploid	47 %	200
A9032	<i>spo13D dmc1D fpr3D</i>	diploid	12 %	200
A9802	<i>spo13D</i>	haploid <sup>1</sup>	20 %	40
A9799	<i>fpr3D</i>	haploid	23 %	40

<sup>1</sup> Both haploid strains harbor *MATa* and *MATa* information to allow them to enter meiosis.

<sup>2</sup> Tetrads and dyads were dissected and viability of individual spores was determined.

**Table S2: Strains**

Strain	Relevant genotype
A1631	<i>MATa</i> , <i>pGAL-GLC7::LEU2</i> (W303)
A1972	<i>MATa/alpha</i> , <i>REC8-3HA::URA3/REC8-3HA::URA3</i>
A5054	<i>MATa</i> , <i>pSTE5-URA3::TRP1</i> , <i>dmc1D::HIS3</i>
A6030	<i>MATa</i> , <i>GLC7-13Myc::KanMX6</i> (W303)
A6683	<i>MATa/alpha</i> , <i>dmc1D::HIS3/dmc1D::HIS3</i> , <i>fpr3D::KanMX/fpr3D::KanMX</i>
A6871	<i>MATa/alpha</i> , <i>dmc1D::HIS3/+</i> , <i>fpr3D::KanMX/+</i>
A6872	<i>MATa/alpha</i> , <i>dmc1D::HIS3/dmc1D::HIS3</i> , <i>fpr3D::KanMX/+</i>
A6924	<i>MATa/alpha</i> , <i>dmc1D::HIS3/+</i> , <i>fpr3D::KanMX/fpr3D::KanMX</i>
A7587	<i>MATa/alpha</i> , <i>dmc1D::HIS3/dmc1D::HIS3</i> , <i>fpr3D::KanMX/fpr3D::KanMX</i> , <i>ZIP1-GFP::URA3/+</i>
A7588	<i>MATa/alpha</i> , <i>dmc1D::HIS3/+</i> , <i>fpr3D::KanMX/fpr3D::KanMX</i> , <i>ZIP1-GFP::URA3/+</i>
A7589	<i>MATa/alpha</i> , <i>dmc1D::HIS3/+</i> , <i>fpr3D::KanMX/+</i> , <i>ZIP1-GFP::URA3/+</i>

A7590	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/+, ZIP1-GFP::URA3/+</i>
A7593	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/fpr3D::TRP1</i>
A7594	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+</i>
A7872	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::KanMX/+, CDC14-3HA/+</i>
A7877	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/fpr3D::KanMX</i>
A7878	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/+, fpr3D::KanMX/fpr3D::KanMX</i>
A7883	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/+, fpr3D::KanMX/+</i>
A7884	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/+</i>
A8339	<i>MATa/alpha, hop2D::HIS3/hop2D::HIS3, fpr3D::TRP1/+</i>
A8342	<i>MATa/alpha, hop2D::HIS3/+, fpr3D::TRP1/+</i>
A8345	<i>MATa/alpha, hop2D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1, fpr4D::KanMX/fpr4D::KanMX</i>
A8360	<i>MATa/alpha, hop2D::HIS3/hop2D::HIS3, fpr3D::TRP1/fpr3D::TRP1</i>
A8596	<i>MATa/alpha, fpr3D::TRP1/fpr3D::TRP1</i>
A8873	<i>MATa, ura3::MATalpha::URA3</i>
A8989	<i>MATa/alpha, rad50S::URA3/rad50S::URA3, fpr3D::KanMX/fpr3D::KanMX</i>
A8990	<i>MATa/alpha, rad50S::URA3/rad50S::URA3, fpr3D::KanMX/+</i>
A9032	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3D::TRP1, spo13D::KanMX/spo13D::KanMX</i>
A9033	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, spo13D::KanMX/spo13D::KanMX</i>
A9034	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1, spo13D::KanMX/spo13D::KanMX</i>
A9035	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, spo13D::KanMX/spo13D::KanMX</i>
A9036	<i>MATa/alpha, zip1D::LYS2/zip1D::LYS2, pch2D::KanMX/pch2D::KanMX, fpr3D::KanMX/+</i>
A9037	<i>MATa/alpha, zip1D::LYS2/zip1D::LYS2, pch2D::KanMX/+, fpr3D::KanMX/+</i>
A9038	<i>MATa/alpha, zip1D::LYS2/zip1D::LYS2, pch2D::KanMX/pch2D::KanMX, fpr3D::KanMX/fpr3D::KanMX</i>
A9119	<i>MATa/alpha, zip1D::LYS2/zip1D::LYS2, pch2D::KanMX/+, fpr3D::KanMX/fpr3D::KanMX</i>
A9602	<i>MATa/alpha, cdc6::pSCC1-3HA-CDC6::KanMX6/cdc6::pSCC1-3HA-CDC6::KanMX6, fpr3D::TRP1/fpr3D::TRP1</i>
A9603	<i>MATa/alpha, cdc6::pSCC1-3HA-CDC6::KanMX6/cdc6::pSCC1-3HA-CDC6::KanMX6, fpr3D::TRP1/+</i>
A9615	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1, spo11-Y135F-HA::URA3/+</i>
A9617	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, spo11-Y135F-HA::URA3/+</i>
A9618	<i>MATa/alpha, rec8D::KanMX/rec8D::KanMX, fpr3D::TRP1/fpr3D::TRP1</i>
A9619	<i>MATa/alpha, rec8D::KanMX/rec8D::KanMX, fpr3D::TRP1/+</i>
A9620	<i>MATa/alpha, rec8D::KanMX/+, fpr3D::TRP1/fpr3D::TRP1</i>
A9621	<i>MATa/alpha, rec8D::KanMX/+, fpr3D::TRP1/+</i>

A9669	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+</i>
A9670	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3D::TRP1</i>
A9671	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+</i>
A9672	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1</i>
A9674	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/FPR3(WT)::URA3</i>
A9675	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(T345A)::URA3</i>
A9676	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(F341Y/D342V)::URA3</i>
A9677	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(W363L)::URA3</i>
A9678	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(F402Y)::URA3</i>
A9679	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(Y386D)::URA3</i>
A9697	<i>MATa/alpha, zip1D::LYS2/+, pch2D::KanMX/+, fpr3D::KanMX/+</i>
A9700	<i>MATa/alpha, zip1D::LYS2/+, pch2D::KanMX/+, fpr3D::KanMX/fpr3D::KanMX</i>
A9723	<i>MATa, cdc6::pSCC1-3HA-CDC6::KanMX6, fpr3D::TRP1, ura3::MATalpha::URA3</i>
A9799	<i>MATa, spo13D::KanMX, fpr3D::TRP1, ura3::MATalpha::URA3</i>
A9802	<i>MATa, spo13D::KanMX, ura3::MATalpha::URA3</i>
A10122	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, CLB3-3HA:KanMX</i>
A10123	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3D::TRP1, CLB3-3HA:KanMX</i>
A10124	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1, CLB3-3HA:KanMX</i>
A10125	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, CLB3-3HA:KanMX</i>
A10272	<i>MATa, spo11D::TRP1, ura3::MATalpha::URA3</i>
A10416	<i>MATa, RAD52-GFP::TRP1</i>
A10842	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/+, fpr3D::KanMX/fpr3D::KanMX, pch2D::KanMX/pch2D::KanMX</i>
A10843	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/+, pch2D::KanMX/pch2D::KanMX</i>
A10844	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/dmc1D::HIS3, fpr3D::KanMX/fpr3D::KanMX, pch2D::KanMX/pch2D::KanMX</i>
A10912	<i>MATa/alpha, his4B::LEU2/his4X::LEU2(Bam)-URA3, arg4-BglII/arg4-Nsp, cdc6::pSCC1-3HA-CDC6::KanMX6/cdc6::pSCC1-3HA-CDC6::KanMX6</i>
A11012	<i>MATa/alpha, mer3D::KanMX/mer3D::KanMX, fpr3D::TRP1/+</i>
A11013	<i>MATa/alpha, mer3D::KanMX/mer3D::KanMX, fpr3D::TRP1/fpr3D::TRP1</i>
A11014	<i>MATa/alpha, mer3D::KanMX/+, fpr3D::TRP1/+</i>
A11015	<i>MATa/alpha, mer3D::KanMX/+, fpr3D::TRP1/fpr3D::TRP1</i>
A11026	<i>MATa/alpha, his4X::LEU2-(Bam)-URA3/his4B::LEU2, arg4-Nsp/arg4-Bgl II, dmc1D::HIS3/+, fpr3D::KanMX/+, pch2D::KanMX/pch2D::KanMX</i>
A11265	<i>MATa/alpha, his4B::LEU2/his4X::LEU2(Bam)-URA3, arg4-BglII/arg4-Nsp, rad50S::URA3/rad50S::URA3, cdc6::pSCC1-3HA-CDC6::KanMX6/cdc6::pSCC1-3HA-CDC6::KanMX6</i>
A11288	<i>MATa, fpr3D::TRP1, ura3::MATalpha::URA3</i>
A11550	<i>MATa, cdc6::pSCC1-3HA-CDC6::KanMX6, ura3::MATalpha::URA3</i>
A11675	<i>MATa/alpha, his4B::LEU2/his4X::LEU2(Bam)-URA3, arg4-BglII/arg4-Nsp, rad50S::URA3/rad50S::URA3</i>
A12006	<i>MATa, cdc6::pSCC1-3HA-CDC6::KanMX6, spo11D::TRP1 ura3::MATalpha::URA3</i>

A12007	<i>MATa, cdc6::pSCC1-3HA-CDC6::KanMX6, dnl4D::TRP1, fpr3D::TRP1, ura3::MATalpha::URA3</i>
A12168	<i>MATa/alpha, cdc6::pSCC1-3HA-CDC6::KanMX6/cdc6::pSCC1-3HA-CDC6::KanMX6, spo11D::TRP1/spo11D::TRP1</i>
A12169	<i>MATa/alpha, spo11D::TRP1/spo11D::TRP1</i>
A12368	<i>MATalpha, pGAL-FPR3::TRP1 (W303)</i>
A12442	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/fpr3D::TRP1, GLC7-13Myc::KanMX/GLC7-13Myc::KanMX</i>
A12443	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3D::TRP1, GLC7-13Myc::KanMX/GLC7-13Myc::KanMX</i>
A12444	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, GLC7-13Myc::KanMX/GLC7-13Myc::KanMX</i>
A12445	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, GLC7-13Myc::KanMX/GLC7-13Myc::KanMX</i>
A12658	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/FPR3(WT)::URA3, GLC7-13Myc::KanMX/+</i>
A12659	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(T345A)::URA3, GLC7-13Myc::KanMX/+</i>
A12660	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(F341Y/D342V)::URA3, GLC7-13Myc::KanMX/+</i>
A12661	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(W363L)::URA3, GLC7-13Myc::KanMX/+</i>
A12662	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(F402Y)::URA3, GLC7-13Myc::KanMX/+</i>
A12663	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/fpr3(Y386D)::URA3, GLC7-13Myc::KanMX/+</i>
A13749	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, leu2::YIplac128::LEU2, [YEP352::URA3]</i>
A13750	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, leu2::YIplac128::LEU2, [YEP352-FPR3::URA3]</i>
A13751	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, YIplac128::LEU2, [YEP352::URA3]</i>
A13752	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, YIplac128::LEU2, [YEP352-FPR3::URA3]</i>
A13753	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, pHOP1-GLC7::LEU2, [YEP352::URA3]</i>
A13754	<i>MATa/alpha, dmc1D::HIS3/+, fpr3D::TRP1/+, pHOP1-GLC7::LEU2, [YEP352-FPR3::URA3]</i>
A13757	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, pHOP1-GLC7::LEU2, [YEP352::URA3]</i>
A13758	<i>MATa/alpha, dmc1D::HIS3/dmc1D::HIS3, fpr3D::TRP1/+, pHOP1-GLC7::LEU2, [YEP352-FPR3::URA3]</i>
NKY1551	<i>MATa/alpha, his4B::LEU2/his4X::LEU2(Bam)-URA3, arg4-BglII/arg4-Nsp (Storlazzi et al., 1995)</i>

## Supplemental References

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