Supplemental Information

A Mec1- and PP4-Dependent Checkpoint Couples Centromere Pairing to Meiotic Recombination

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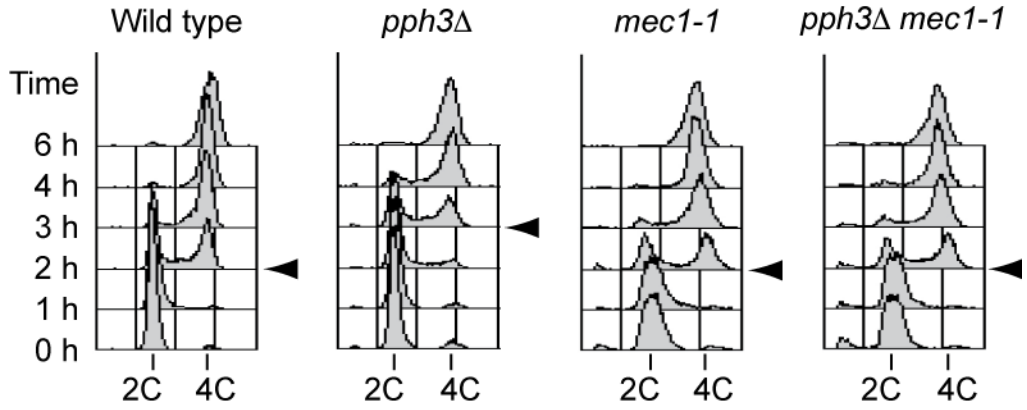

Figure 1 (related to Figure 1). Mec1-dependent replication delay of *pph3Δ* mutants
FACS DNA content analysis of synchronous meiotic cultures of wild type (NKY3230), *pph3Δ* (H2530), *mec1-1 sml1Δ* (H3661), and *pph3Δ mec1-1 sml1Δ* (H3688). Arrowheads indicate time points when cells with 4C DNA content first became detectable.

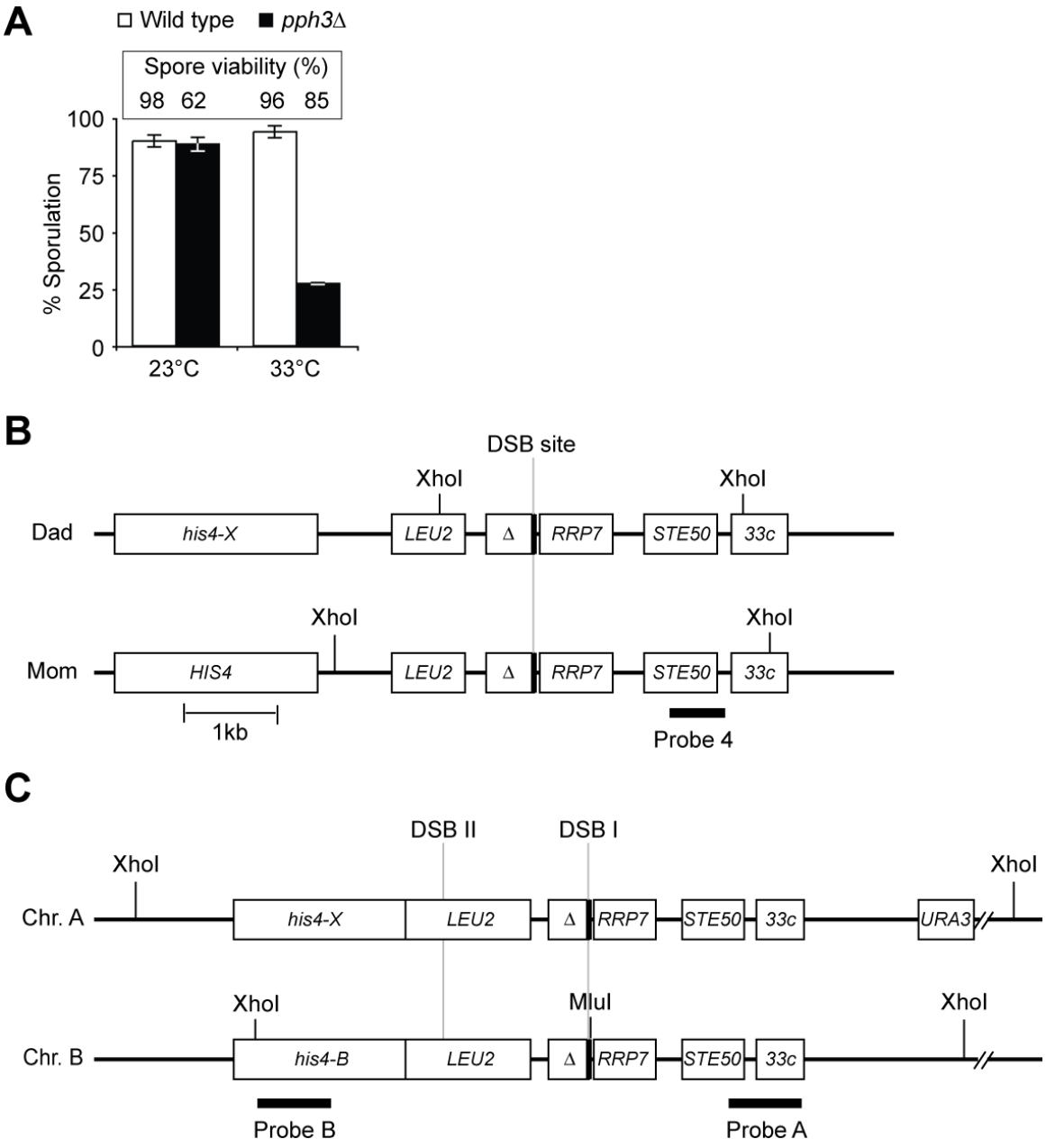

Figure S2 (related to Figure 2). Spore viability and CO/NCO assays systems
(A) Sporulation efficiency and tetrad spore viability of wild-type (Y1678) and *pph3Δ* (Y1675) strains at 23°C and 33°C. At least 300 tetrads were dissected for each condition. Bars represent the standard error of the mean. An increase in viable spores at high temperatures is a phenotype shared with *msh4Δ* and *msh5Δ* mutants (Chan et al., 2009). (B, C) Schematics of the *HIS4LEU2* constructs used in Figure 2A-F and 2G, H, respectively. Adapted from (Hunter and Kleckner, 2001; Storlazzi et al., 1995).

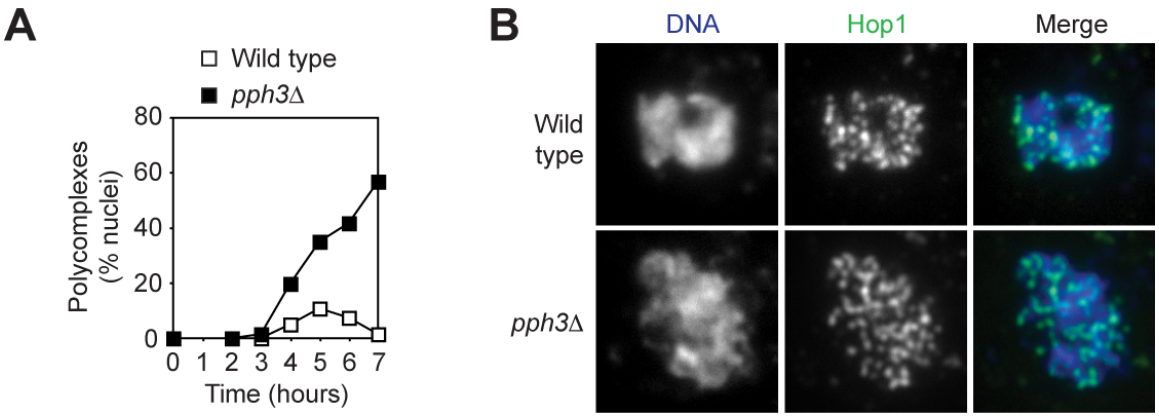

Figure S3 (related to Figure 4). Polycomplexes and chromosome axes in *pph3Δ* mutants

(A, B) Wild-type (A4962) and *pph3Δ* (H2086) strains were induced to undergo synchronous meiosis and nuclei were analyzed by immunofluorescence on chromosome spreads. (A) Polycomplex formation was quantified from the same experiment shown in Figure 4A-C. Examples of the brightly staining Zip1 polycomplexes can be seen in Figure 4B. (B) Chromosomes were spread at the 4h time point and analyzed by immunofluorescence against Hop1 (green). DNA was stained with DAPI (blue).

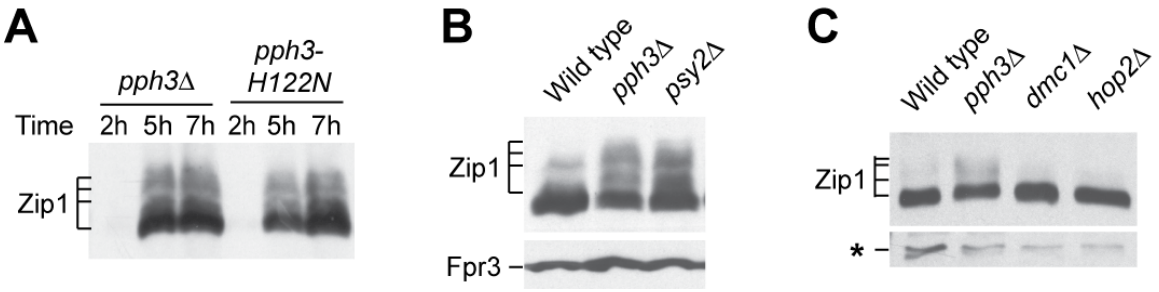

Figure S4 (related to Figure 5). Zip1 phosphoshift in *pp4* mutants and meiotic repair mutants

(A) Cells carrying a full deletion (H2086) or a mutation coding for a H122N amino acid change in the catalytic site of Pph3 (H3354) were induced to undergo synchronous meiosis. Zip1 was analyzed by Western blotting at the indicated time points. (B, C) Western blot analysis of Zip1 4 hours after meiotic induction. (B) Wild type (A4962), *pph3Δ* (H2086), and *psy2Δ* (H2548). Fpr3 was used as loading control. (C) Wild type (A4962), *pph3Δ* (H2086), *dmc1Δ* (H3260) and *hop2Δ* (H5000). We reproducibly observed increased levels of Zip1 in *dmc1Δ* and *hop2Δ* mutants. To permit comparison of Zip1 phosphorylation levels, total sample volume loaded in the *dmc1Δ* and *hop2Δ* lanes was 50% of the wild type and *pph3Δ* lanes. Star indicates a cross-reacting band used as loading control.

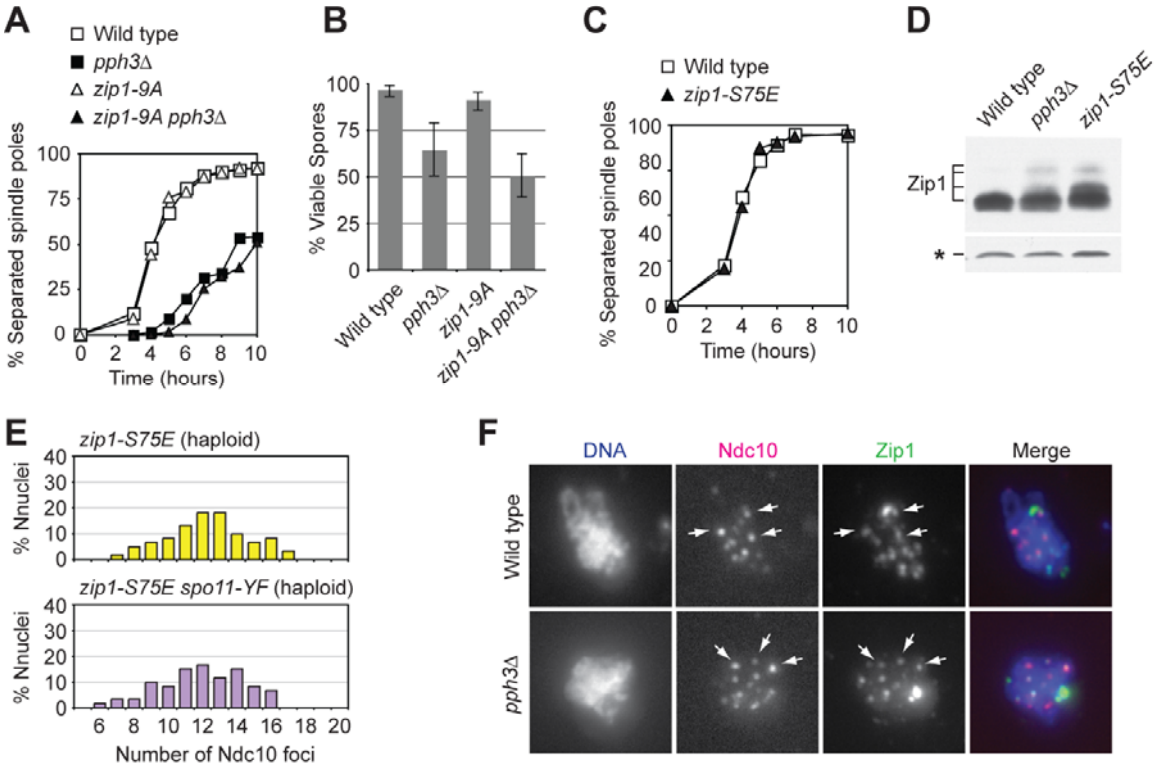

Figure S5 (related to Figure 7). Meiotic phenotypes of Zip1 point mutants and centromeric localization of Zip1

(A) Wild-type (H4025), *pph3Δ* (H4024), *zip1-9A* (H4346), and *zip1-9A pph3Δ* (H4341) strains were induced to undergo synchronous meiosis at 33°C and spindle pole separation was determined by tubulin staining. (B) Spore viability levels were determined for the same strains by tetrad dissection. Bars indicate standard deviation. More than 200 tetrads were analyzed for each strain. (C) Wild-type (H4617) and *zip1-S75E* (H4700) strains were induced to undergo synchronous meiosis and spindle pole separation was determined by tubulin staining. (D) Zip1 was analyzed by Western blotting of wild-type (H4025), *pph3Δ* (H4024) and *zip1-S75E* (H4700), 3.5 hours after meiotic induction. (E) Analysis of Ndc10 focus number in *MATa/alpha* haploid *zip1-S75E* (H4729) and *zip1-S75E spo11-Y135F* (H5025) cells carrying Ndc10-6HA. Nuclei were spread 4 hours after meiotic initiation and stained for Ndc10-6HA. 60 spread nuclei were analyzed for each strain. (F) Analysis of centromeric localization of Zip1 on meiotic spreads of *MATa/alpha* haploid wild-type (H4277) and *pph3Δ* (H4255) cells carrying Ndc10-6HA. Samples were taken 4 hours after meiotic induction and stained for Zip1 (green), Ndc10 (red), and DNA (blue).

Table S2. Strains used in this study

Strain	Relevant genotype	Reference
A4962	*MATa/alpha, ho::LYS2/ho::LYS2, lys2/lys2, ura3/ura3, trp1::hisG/trp1::hisG, his3::hisG/his3::hisG, leu2::hisG/leu2::hisG*	(Lee and Amon, 2003)
H642	A4962 but *spo11-Y135F-HA::URA3/spo11-Y135F-HA::URA3*	This study
H1506	A4962 but *zip1Δ::LYS2/zip1Δ::LYS2*	This study
H2086	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2*	This study
H2118	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, spo11-Y135F-HA::URA3/spo11-Y135F-HA::URA3*	This study
H2157	NKY1551 but *pph3Δ::LEU2/pph3Δ::LEU2*	This study
H2530	NKY3230 but *pph3Δ::LEU2/pph3Δ::LEU2*	This study
H2548	A4962 but *psy2Δ::URA3/psy2Δ::URA3*	This study
H2561	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, mec1-1/mec1-1, sml1Δ::KanMX6/ sml1Δ::KanMX6*	This study
H3206	A4962 but *ZIP4-6HA::KanMX6/+*	This study
H3241	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, ZIP4-6HA::KanMX6/+*	This study
H3260	A4962 but *dmc1Δ::HIS3/dmc1Δ::HIS3*	This study
H3313	NKY1551 but *psy2Δ::URA3/psy2Δ::URA3*	This study
H3354	A4962 but *pph3Δ-H122N/pph3Δ-H122N*	This study
H3661	NKY3230 but *mec1-1/mec1-1, sml1Δ::KanMX6/ sml1Δ::KanMX6*	This study
H3688	NKY3230 but *pph3Δ::LEU2/pph3Δ::LEU2, mec1-1/mec1-1, sml1Δ::KanMX6/ sml1Δ::KanMX6*	This study
H3727	A4962 but *zip3Δ::KanMX6/zip3Δ::KanMX6*	This study
H3922	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip1Δ::URA3/zip1Δ::URA3*	This study
H3925	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, mek1Δ::KanMX4/mek1Δ::KanMX4, ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H3928	A4962 but *ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H3929	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H3930	A4962 but *zip2Δ::HIS3/zip2Δ::HIS3*	This study
H3957	A4962 but *spo16Δ::TRP1/spo16Δ::TRP1*	This study

H3993	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip2Δ::HIS3/zip2Δ::HIS3*	This study
H3994	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, spo16Δ::TRP1/spo16Δ::TRP1*	This study
H3997	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip3Δ::KanMX6/zip3Δ::KanMX6*	This study
H4017	A4962 but *msh4::URA3/msh4::URA3*	This study
H4024	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip1Δ::URA3/zip1Δ::URA3, trp1::ZIP1::TRP1/ trp1::ZIP1::TRP1*	This study
H4025	A4962 but *zip1Δ::URA3/zip1Δ::URA3, trp1::ZIP1::TRP1/ trp1::ZIP1::TRP1*	This study
H4031	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, mec1-1/mec1-1, sml1Δ::KanMX6/ sml1Δ::KanMX6, ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H4069	A4962 but *PSY2-13MYC::TRP1/PSY2-13MYC::TRP1, NDC10-6HA::HIS3MX6/ NDC10-6HA::HIS3MX6*	This study
H4070	A4962 but *msh5Δ::HIS3/msh5Δ::HIS3*	This study
H4071	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, msh5Δ::HIS3/msh5Δ::HIS3*	This study
H4087	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, spo11-Y135F-HA::URA3/spo11-Y135F-HA::URA3, ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H4096	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, msh4::URA3/msh4::URA3*	This study
H4176	A4962 but *zip4Δ::HIS3/zip4Δ::HIS3*	This study
H4177	A4962 but *mer3Δ::KanMX4/mer3Δ::KanMX4*	This study
H4178	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, mer3Δ::KanMX4/mer3Δ::KanMX4*	This study
H4181	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip1Δ::URA3/zip1Δ::URA3, trp1::zip1-8A::TRP1/ trp1::zip1-8A::TRP1*	This study
H4184	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip4Δ::HIS3/zip4Δ::HIS3*	This study
H4189	H4277 but *spo11-Y135F-HA::URA3*	This study
H4190	H4277 but *pph3Δ::LEU2, spo11-Y135F-HA::URA3*	This study
H4255	H4277 but *pph3Δ::LEU2*	This study
H4277	*MATa, ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::MATalpha::TRP1, NDC10-6HA::HIS3*	This study
H4341	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip1Δ::URA3/zip1Δ::URA3, trp1::zip1-9A::TRP1/ trp1::zip1-9A::TRP1*	This study
H4346	A4962 but *zip1Δ::URA3/zip1Δ::URA3, trp1::zip1-9A::TRP1/ trp1::zip1-9A::TRP1*	This study

H4412	H4277 but *pph3Δ::LEU2, mec1-1, sml1Δ::KanMX6*	This study
H4445	H4446 but *pph3Δ::LEU2/pph3Δ::LEU2*	This study
H4446	*MATa/alpha, ho::hisG/ho::hisG, ura3(Δsma-pst::hisG)/ura3(Δsma-pst::hisG), leu2::hisG/leu2::hisG, HIS4::LEU2-(NBam;ori)/his4X::LEU2-(NgoMIV)-URA3, zip1Δ::URA3/zip1Δ::URA3, trp1::ZIP1::TRP1/ trp1::ZIP1::TRP1*	This study
H4462	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, zip1Δ::URA3/zip1Δ::URA3, trp1::zip1-S75A::TRP1/ trp1::zip1-S75A::TRP1*	This study
H4568	H4277 but *mec1-1, sml1Δ::KanMX6*	This study
H4600	*MATa, ho::hisG, ura3, leu2::hisG, his3::hisG,* lys2::MATalpha::LYS2, *pph3Δ::LEU2, zip1Δ::URA3, trp1::zip1-S75A::TRP1, NDC10-6HA::HIS3*	This study
H4617	A4962 but *zip1Δ::URA3/zip1Δ::URA3, trp1::ZIP1::TRP1/ trp1::ZIP1::TRP1*	This study
H4700	A4962 but *zip1Δ::URA3/zip1Δ::URA3, trp1::zip1-S75E::TRP1/ trp1::zip1-S75E::TRP1*	This study
H4729	*MATa, ho::hisG, ura3, leu2::hisG, his3::hisG,* lys2::MATalpha::LYS2, *zip1Δ::URA3, trp1::zip1-S75E::TRP1, NDC10-6HA::HIS3*	This study
H5000	A4962 but *hop2Δ::HIS3/hop2Δ::HIS3*	This study
H5010	A4962 but *pph3Δ::LEU2/pph3Δ::LEU2, mec1-1/mec1-1, sml1Δ::KanMX6/ sml1Δ::KanMX6, tel1Δ::HIS3/tel1Δ::HIS3, ndt80Δ::TRP1/ndt80Δ::TRP1*	This study
H5025	H4729 but *spo11-Y135F-HA::URA3*	This study
H5206	H4446 but *trp1::zip1-S75A::TRP1/ trp1::zip1-S75A::TRP1, pph3Δ::LEU2/pph3Δ::LEU2*	This study
H5207	H4446 but *trp1::zip1-S75A::TRP1/ trp1::zip1-S75A::TRP1, pph3Δ::LEU2/+*	This study
H5208	H4446 but *trp1::zip1-S75E::TRP1/ trp1::zip1-S75E::TRP1*	This study
NKY1551	*MATa/alpha, ho::LYS2/ho::LYS2, lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, arg4-Bgl/arg4-Nsp, his4B::LEU2/his4X::LEU2(Bam)-URA3*	(Storlazzi et al., 1995)
NKY3230	*MATa/alpha, ho::hisG/ho::hisG, ura3(Δsma-pst::hisG)/ura3(Δsma-pst::hisG), leu2::hisG/leu2::hisG, HIS4::LEU2-(NBam)/his4X::LEU2-(NBam)-URA3*	(Hunter and Kleckner, 2001)
Y1675	Y1678 but *pph3Δ::KanMX4/pph3Δ::KanMX4*	This study
Y1678	*MATa/alpha, his4-B/+, leu2-R/+, ade2/ade2, CEN3::ADE2/CEN3, trp5-S/+, cyh2-R/+, met13-B/+, lys5-P/+, arg4-Bgl/+, thr1-A/+, CEN8::URA3/CEN8, ura3(Δsma-pst::hisG)/ura3(Δsma-pst::hisG), cup1-S/+*	(Martini et al., 2006)

Y1683	Y1678 but *msh4Δ::hphMX4*/*msh4Δ::hphMX4*	This study
Y1735	Y1678 but *pph3Δ::KanMX4*/*pph3Δ::KanMX4*, *msh4Δ::hphMX4*/*msh4Δ::hphMX4*	This study

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast strains and plasmids

All strains are derivatives of SK1 and are listed in Supplemental Table 2. Gene deletions and epitope tags were constructed using PCR-based gene replacement and tagging techniques described in (Longtine et al., 1998). Pph3-H122N was created in situ using the delitto perfetto method (Storici et al., 2001). *zip1-S75A* and *zip1-S75E* were created by site-directed mutagenesis (QuikChange II, Stratagene) of pHSS6 (Sym et al., 1993). To create the *zip1-8A* and *zip1-9A* mutations, an AatII/EcoRV fragment of *ZIP1* containing the last 7 S/TQ sites mutated to AQ (synthesized by Mr. Gene GmbH) was cloned into pHSS6. The remaining two S/TQ sites were mutated to AQ by site-directed mutagenesis. All *zip1* constructs were integrated at the *TRP1* locus. The *zip1-9A* point mutant has the following mutations: S75A, T187A, T434A, S473A, S515A, S546A, S593A, S726A and T754A.

DNA content analysis

DNA content analysis was performed as described in (Bell et al., 1993). Briefly, cells from 150µl of meiotic culture were fixed overnight in 70% ethanol. Cell pellets were resuspended in 500µl 50mM sodium citrate containing 20µg/ml RNaseA (Sigma) and incubated at 50°C for 2 hours. 5µl of proteinase K (20µg/ml, Amresco) was added and samples were incubated at 50°C for another 2 hours. Finally, samples were mixed with 500µl 50mM sodium citrate containing 2µM Sytox Green (Invitrogen). Samples were briefly sonicated and analyzed by FACS.

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