SUPPLEMENTAL DATA

Functional impact of the H2A.Z histone variant during meiosis in *Saccharomyces cerevisiae*

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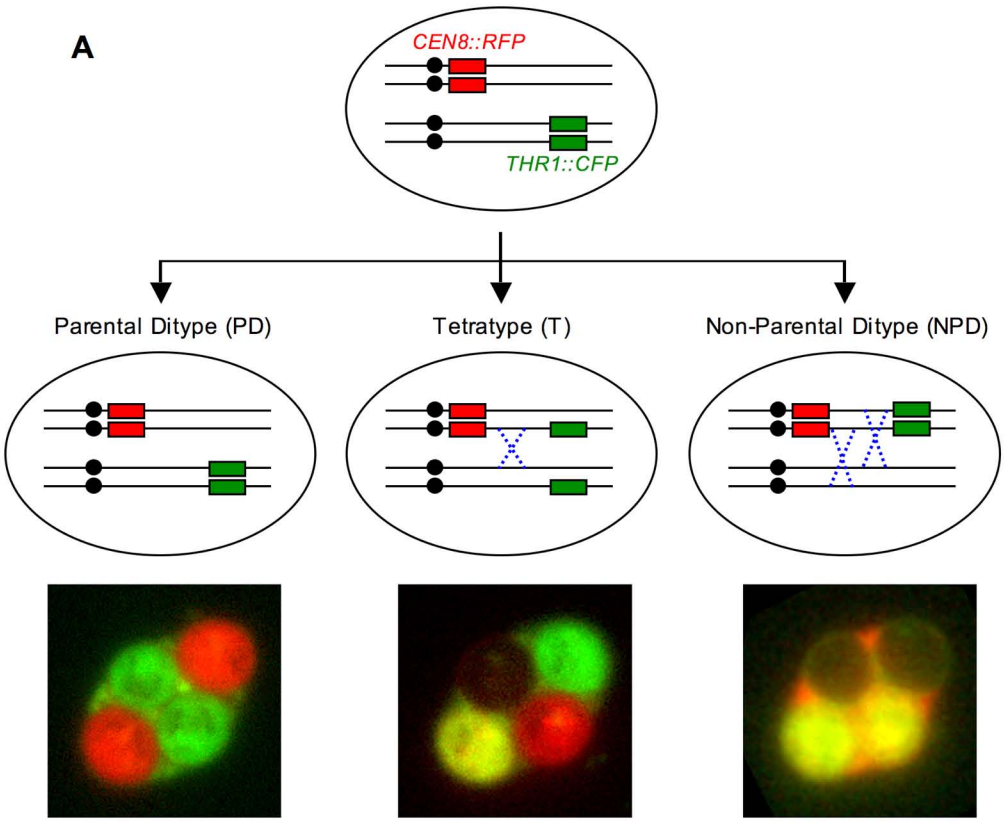
Supplemental Figures (Figures S1-S8)

Table S1 (Strains list)

Table S2 (Plasmids list)

Table S3 (Antibodies list)

A



B

	PD(%)	T(%)	NPD(%)	Genetic distance (cM)	n
wild type	61.62	38.00	0.37	20.11	2245
htz1	55.92	43.72	0.38	22.98	1027
mer3	73.71	16.13	0	8.91	302

Figure S1. Analysis of crossover frequency by spore-autonomous fluorescence assay. (A) Cartoon depicting the potential configuration of the fluorescent reporter markers on Chromosome VIII and representative images of the different types of asci, according to (Thacker *et al.* 2011). Dashed crosses represent possible recombination events leading to each configuration. (B) The table shows the frequency of PD, T and NPD tetrads, map distance expressed in centiMorgan (cM) and the number of tetrads scored (n) pooled from two independent experiments. Strains are DP969 (wild type), DP973 (*htz1*) and DP974 (*mer3*).

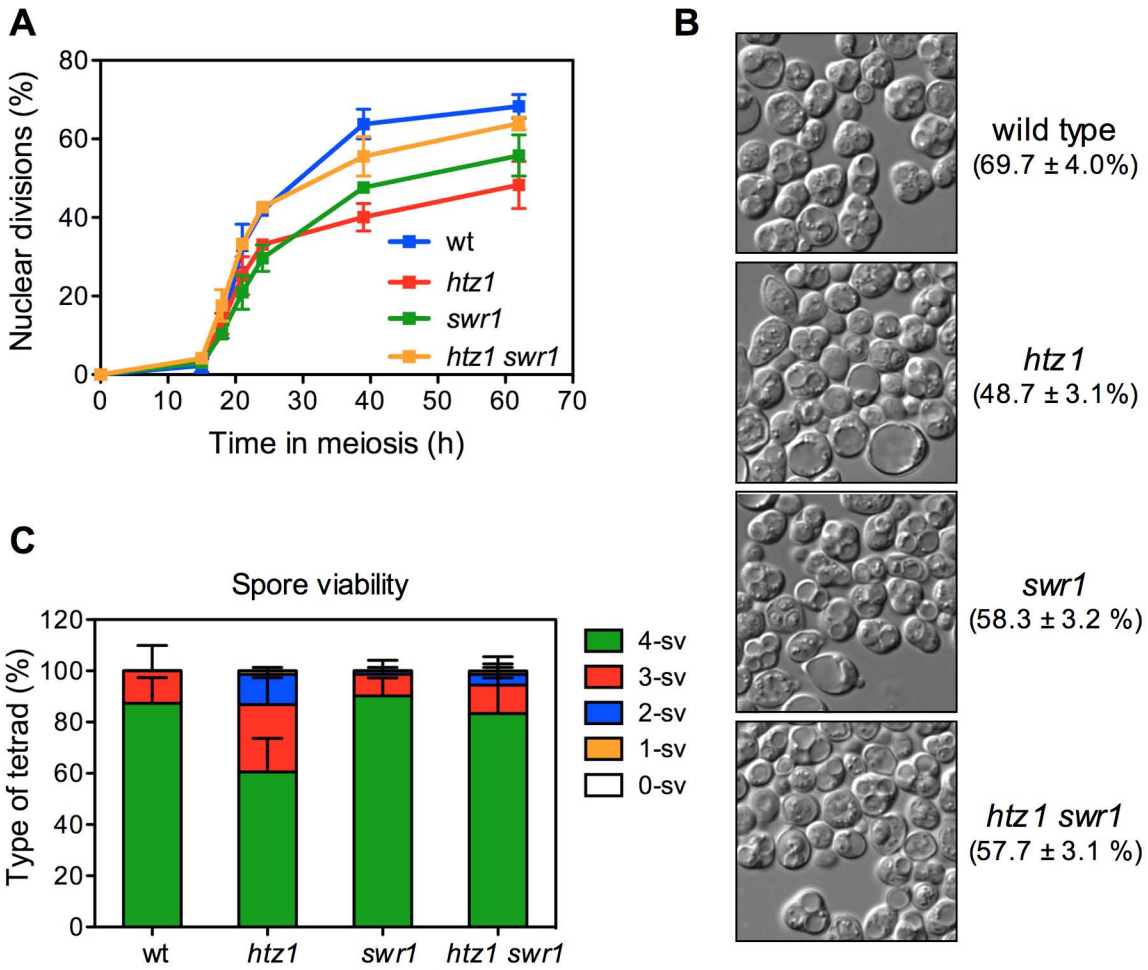


Figure S2. Meiotic impact of the SWR1 co mplex in the p resence or absence of H2A.Z. (A) Time course analysis of meiotic nuclear divisions; the percentage of cells containing two or more nuclei is represented. Error bars: range; n=2. (B) Representative DIC images of asci. The sporulation frequency after 62 hours in liquid sporulation medium is shown in parentheses. (C) Spore viability determined by tetrad dissection. The distribution of tetrad types as the percentage of tetrads with 4, 3, 2, 1 and 0 viable spores (4-sv, 3-sv, 2-sv, 1-sv and 0-sv, respectively) is represented. At least 288 spores were scored for each strain Error bars: range; n=2. Strains are DP421 (wild type), DP630 (*htz1*), DP1174 (*swr1*) and DP1056 (*htz1 swr1*).

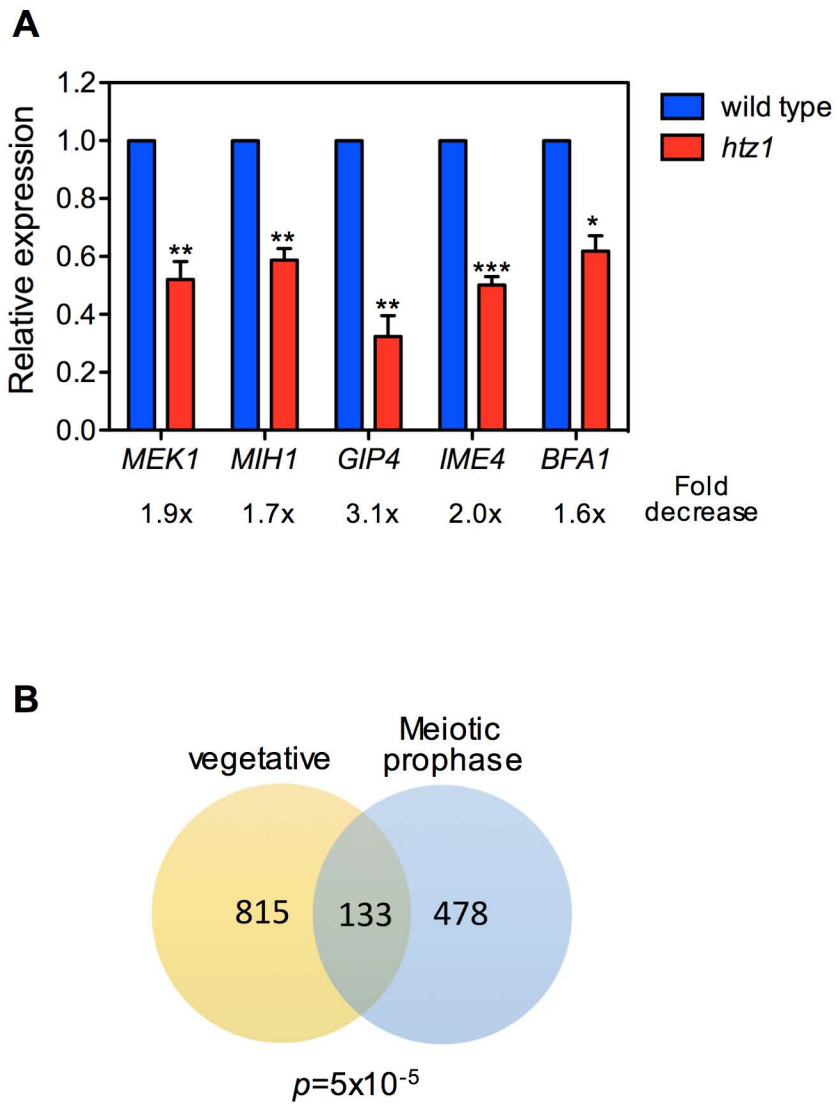


Figure S3. Altered meiotic gene expression in the *htz1* mutant. (A) RT-PCR analysis of mRNA levels of the indicated genes at 15 hours in meiosis. The graph shows relative levels in *htz1* normalized to those in the wild type. Error bars: SD; n=3 (except for *BFA1*; n=2). (B) Venn diagram showing the number of overlapping genes misregulated by *htz1* (1.5-fold cutoff) in vegetative and meiotic cells (15 h). The data for vegetative cells was obtained from (Morillo-Huesca *et al.* 2010). The *p* value was calculated by a hypergeometric test. Strains are DP421 (wild type) and DP1016 (*htz1*).

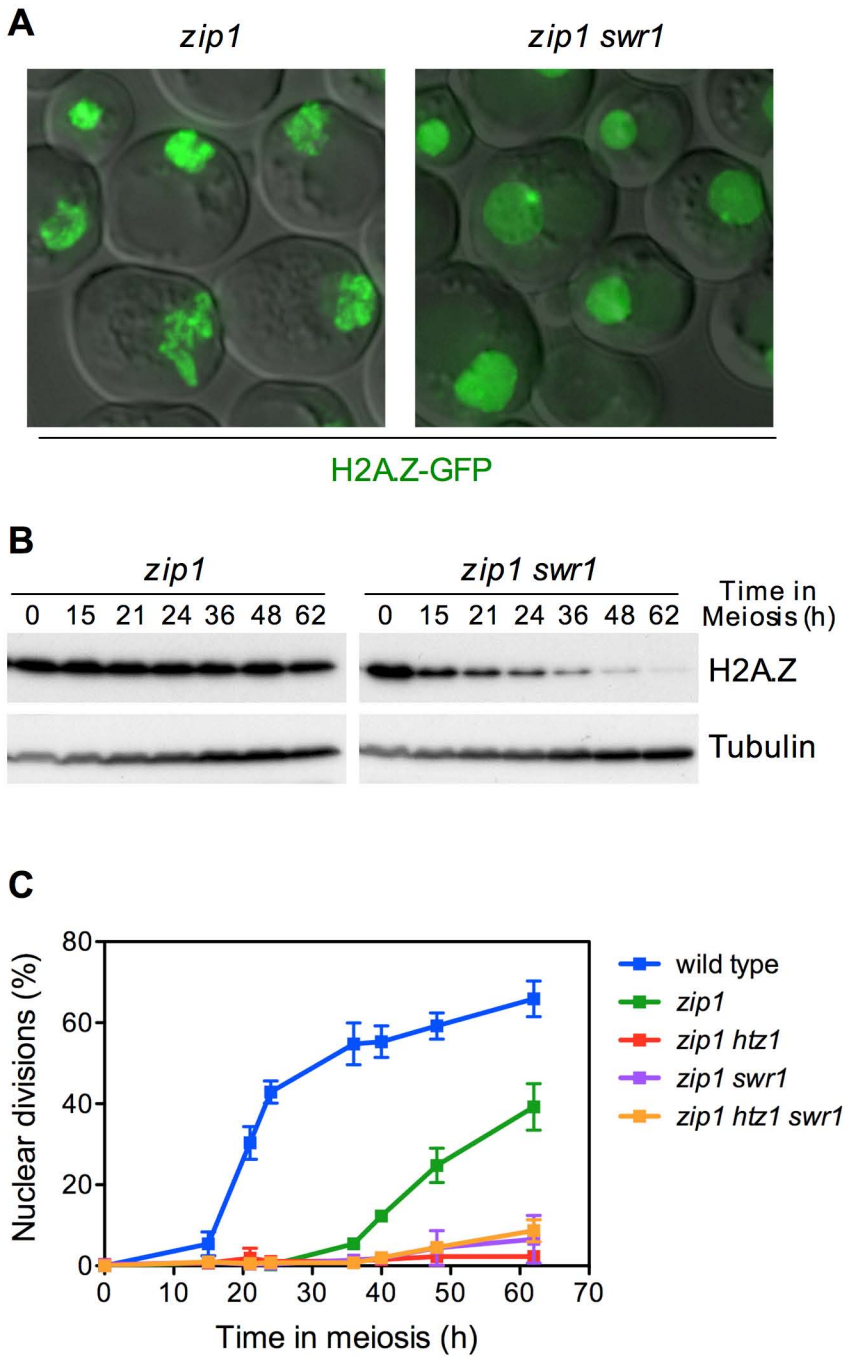


Figure S4. SWR1-dependent chromatin incorporation of H2A.Z is required for normal *zip1*-induced meiotic checkpoint response. (A) Representative images of *zip1* and *zip1 swr1* cells expressing *HTZ1-GFP* at 15 hours after meiotic induction. (B) Western blot analysis of H2A.Z production during meiosis detected with anti-GFP antibodies. Tubulin was used as a loading control. Strains in (A-B) are DP839 (*zip1 HTZ1-GFP*) and DP842 (*zip1 swr1 HTZ1-GFP*). (C) Time course analysis of meiotic nuclear divisions; the percentage of cells containing two or more nuclei is represented. Error bars: SD; n=3. Strains are DP421 (wild type), DP422 (*zip1*), DP776 (*zip1 htz1*), DP804 (*zip1 swr1*) and DP777 (*zip1 swr1 htz1*).

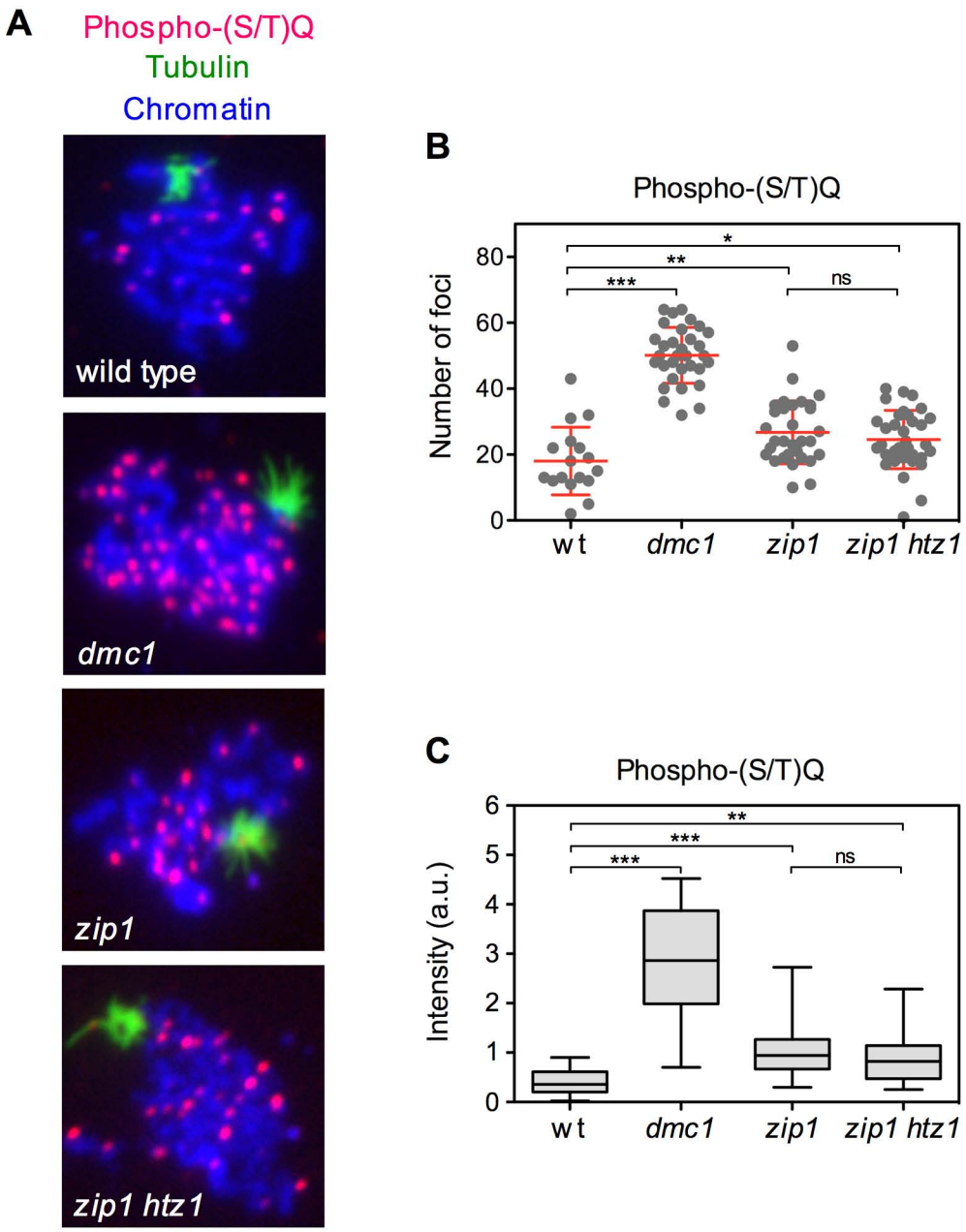


Figure S5. The *zip1 htz1* mutant does not sustain additional Mec1/Tel1-dependent DNA damage signaling compared to *zip1*. (A) Immunofluorescence of prophase meiotic chromosomes stained with DAPI (blue), and anti-phospho-(S/T)-Q (red) and anti-tubulin (green) antibodies. The *dmc1* mutant was included as a positive control for the accumulation of extensive meiotic DNA damage (unrepaired resected DSBs). Representative nuclei are shown. (B) Quantification of the number of phospho-(S/T)-Q foci per nucleus. Error bars: SD. (C) Quantification of the fluorescence intensity of the phospho-(S/T)-Q signal per nucleus. Whiskers in the box plot represent the maximum to minimum values. The strains used and the number of nuclei scored in (B-C) are: DP421 (wild type; n=17), DP590 (*dmc1*; n=32), DP1525 (*zip1*; n=31) and DP1526 (*zip1 htz1*; n=34).

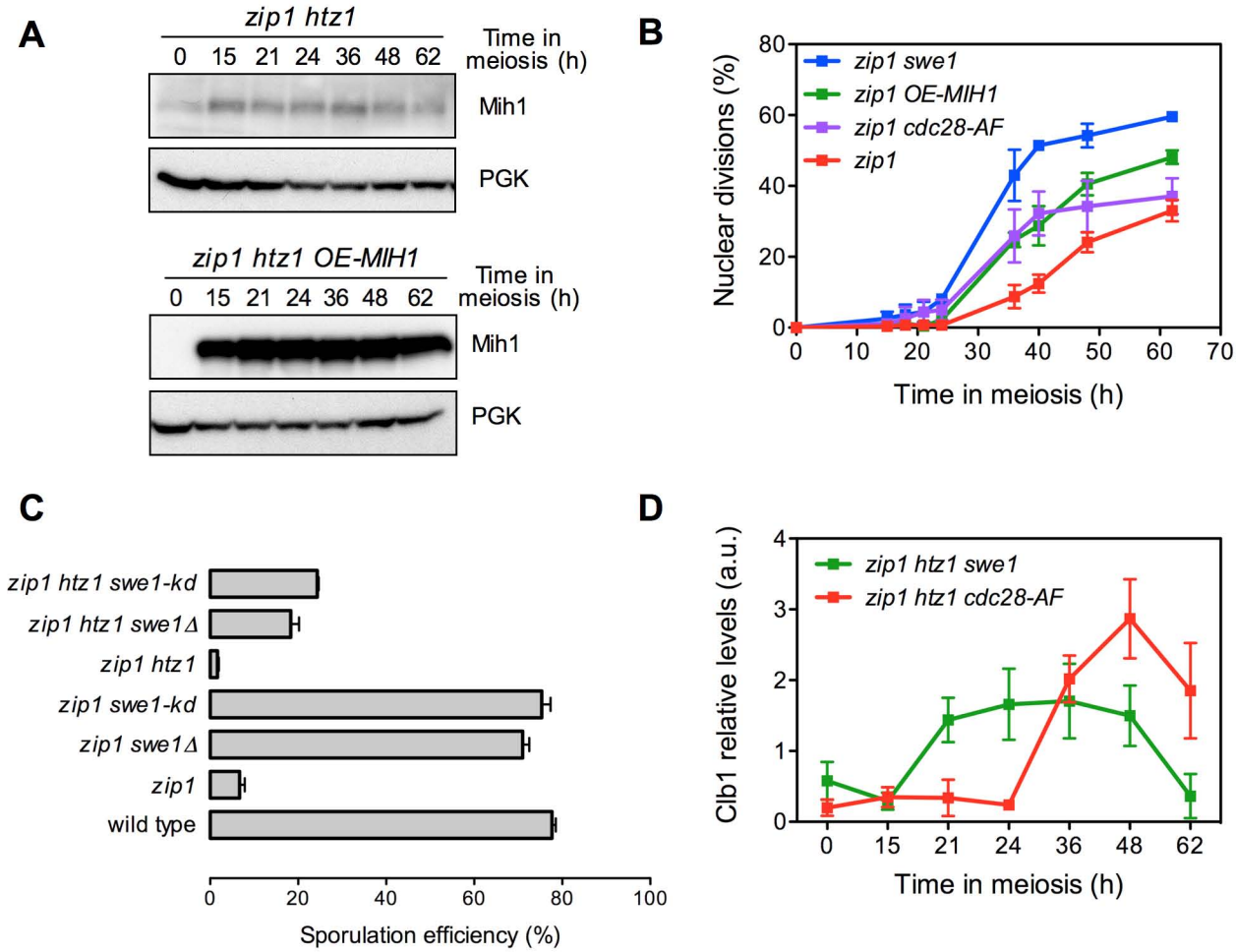


Figure S6. Additional analysis of the role of CDK inhibitory phosphorylation in the meiotic checkpoint. (A) Western blot showing *MIH1-GFP* overexpression from the *HOP1* promoter in a high-copy plasmid. The DP1134 strain *(zip1 htz1 MIH1-GFP*) was transformed with vector alone (left panels) or with the pSS265 plasmid (right panels). PGK was used as a loading control. Mih1 was detected with anti-GFP antibodies. (B) Faster meiotic progression *in zip1 cdc28-AF* and *zip1 OE-MIH1* compared to *zip1*. Time course analysis of meiotic nuclear divisions; the percentage of cells containing two or more nuclei is represented. Strains are DP1157 (*zip1 swe1*), DP1153 (*zip1 cdc28-AF*) and DP422 transformed with empty vector pSS248 (*zip1*) or with pSS265 (*zip1 OE-MIH1*). Error bars: SD; n=3. (C) The kinase-dead *swe1-N584A* mutant (*swe1-kd*) phenocopies *SWE1* deletion. The sequence changes introduced to generate the *swe1-N584A* mutation in the genomic locus by *delitto perfetto* are shown. The graph represents the sporulation efficiency determined by microscopic counting as the percentage of cells forming mature or immature asci after 3 days on sporulation plates. Error bars: SD; n=3. Strains are: DP1353 (wild type), DP1354 (*zip1*), DP1157 (*zip1 swe1*) and DP1467 (*zip1 swe1-kd*), DP1414 (*zip1 htz1*), DP1113 (*zip1 htz1 swe1*) and DP1468 (*zip1 htz1 swe1-kd*). (D) Quantification of Clb1 levels, normalized to PGK, throughout meiosis in the indicated strains. Error bars: SD; n=3. Strains are DP1113 (*zip1 htz1 swe1*) and DP1416 (*zip1 htz1 cdc28-AF*).

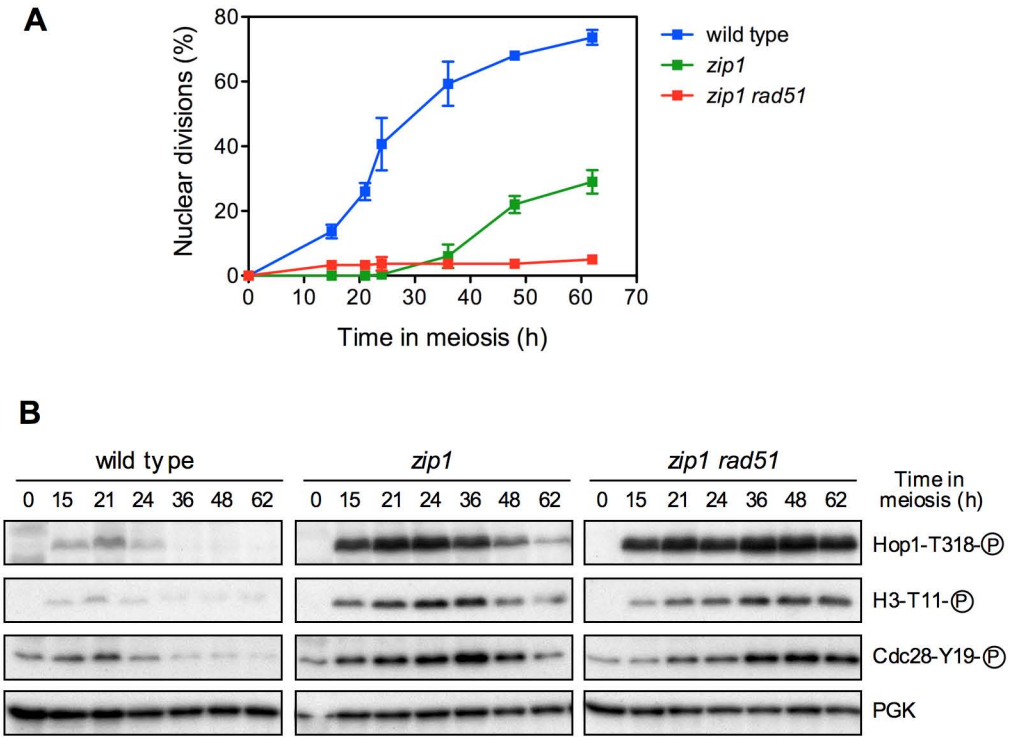


Figure S7. Deletion of *RAD51* leads to sustained checkpoint activation and meiotic arrest in *zip1*. (A) Time course analysis of meiotic nuclear divisions; the percentage of cells containing two or more nuclei is represented. Error bars: SD; n=3. (B) Western blot analysis of the indicated molecular markers of checkpoint activity. PGK was used as a loading control. Strains are DP1359 (wild type), DP1360 (*zip1*) and DP1364 (*zip1 rad51*).

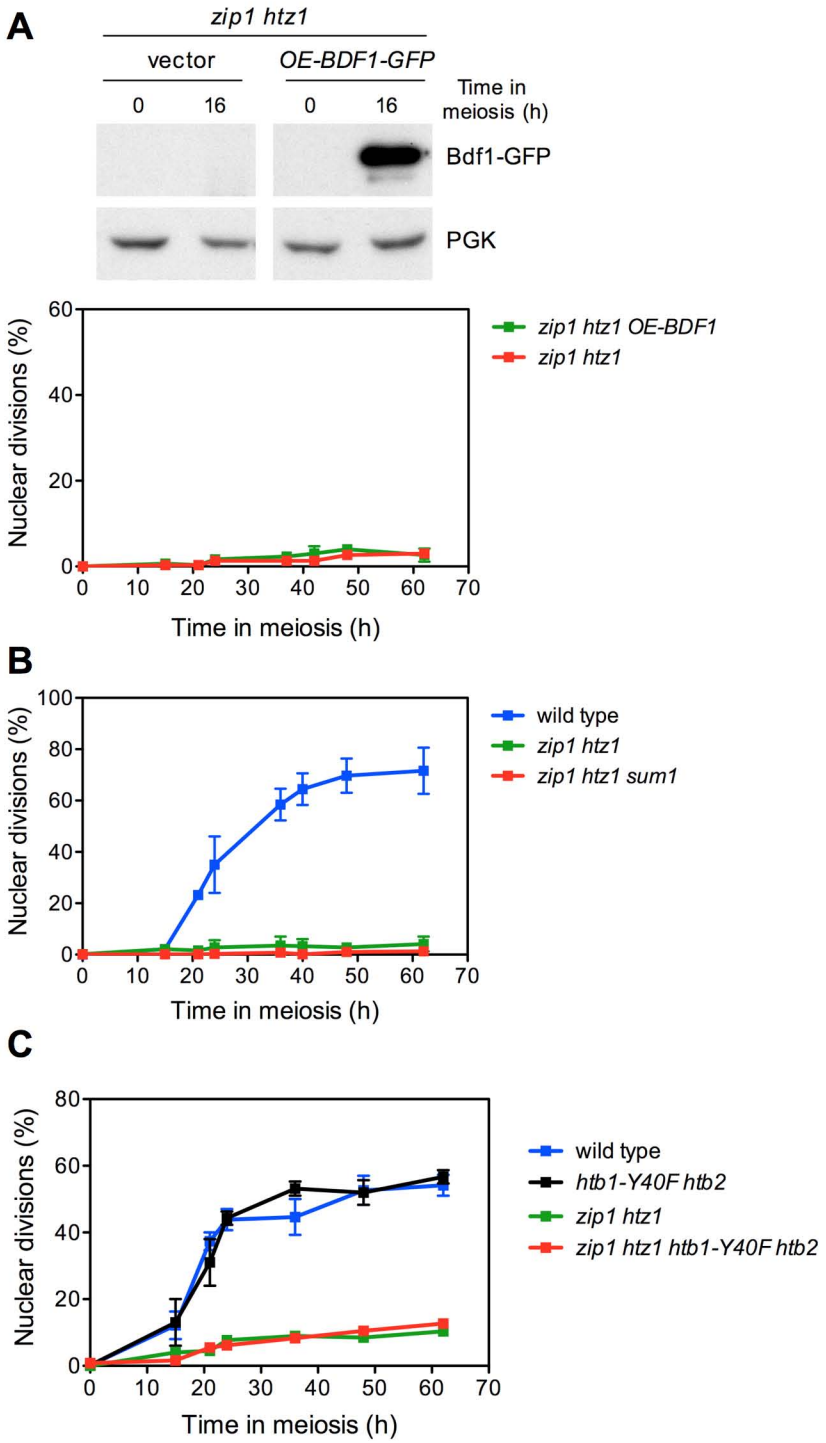


Figure S8. Meiosis-specific overexpression of *BDF1*, deletion of *SUM1* and mutation of *H2B-Y40* do not suppress *zip1 htz1* arrest. (A) Upper panel; western blot analysis of *zip1 htz1* cells (DP1017) transformed with vector (pSS248) or with a 2-micron plasmid expressing *BDF1-GFP* from the *HOP1* promoter (*OE-BDF1-GFP*; pSS354). Extracts were prepared at time zero or 16 hours after meiotic induction (around the peak of prophase) and analyzed with anti-GFP antibodies. PGK was used as a loading control. Lower panel; time course analysis of meiotic nuclear divisions in the same strains. The percentage of cells containing two or more nuclei is represented. Error bars: SD; n=3. (B) Time course analysis of meiotic nuclear divisions in the indicated strains. Wild type (DP421), *zip1 htz1* (DP630) and *zip1 htz1 sum1* (DP1441). Error bars: range; n=2. (C). Time course analysis of meiotic nuclear divisions in the indicated strains. Wild type (DP1445), *htb1-Y40F htb2* (DP1446), *zip1 htz1* (DP1449) and *zip1 htz1 htb1-Y40F htb2* (DP1450). Error bars: range; n=2.

Table S1. *Saccharomyces cerevisiae* strains

Strain	Genotype	Source
DP421	*MAT***a**/*MATα leu2,3-112 his4-260 thr1-4 ura3-1 trp1-289 ade2-1 lys2ΔNheI*	PSS Lab
DP422	DP421 *zip1::LYS2*	PSS Lab
DP437	DP421 *ZIP1-GFP*	PSS Lab
DP449	DP421 *zip1::LYS2 DDC2-GFP::TRP1*	PSS Lab
DP590	DP421 *dmc1::hphMX4 p306(BrdU-Inc)::URA3/ura3-1*	PSS Lab
DP630	DP421 *htz1::URA3*	This work
DP631	DP421 *zip1::LYS2 htz1::URA3*	This work
DP713	DP421 *mek1::kanMX6*	PSS Lab
DP776	DP421 *zip1::LYS2 htz1::URA3 DDC2-GFP::TRP1*	This work
DP777	DP421 *zip1::LSY2 htz1::URA3 swr1::natMX4 DDC2-GFP::TRP1*	This work
DP804	DP421 *swr1::natMX4 zip1::LSY2 DDC2-GFP::TRP1*	This work
DP815	DP421 *zip1::LYS2 htz1::URA3 spo11::natMX4 DDC2-GFP::TRP1*	This work
DP816	DP421 *zip1::LYS2 htz1::URA3 ddc2::TRP1 sml1::KanMX6*	This work
DP838	DP421 *ZIP1-GFP htz1::URA3*	This work
DP839	DP421 *zip1::LYS2 HTZ1-GFP::kanMX6*	This work
DP840	DP421 *HTZ1-GFP::kanMX6*	This work
DP841	DP421 *swr1::natMX4 HTZ1-GFP::kanMX6*	This work
DP842	DP421 *zip1::LYS2 swr1::natMX4 HTZ1-GFP::kanMX6*	This work
DP969	SK1 *CEN8::tdTomato-LEU2/CEN8 THR1:mCerulean-TRP1/THR1*	S. Keeney
DP973	SK1 *CEN8::tdTomato-LEU2/CEN8 THR1:mCerulean-TRP1/THR1 htz1::hphMX4*	This work
DP974	SK1 *CEN8::tdTomato-LEU2/CEN8 THR1:mCerulean-TRP1/THR1 mer3::hphMX4*	This work
DP1016	DP421 *htz1::hphMX4*	This work
DP1017	DP421 *zip1::LYS2 htz1::hphMX4*	This work
DP1056	DP421 *htz1::URA3 swr1:: natMX4*	This work
DP1113	DP421 *zip1::LYS2 htz1::hphMX4 swe1::natMX4*	This work
DP1134	DP421 *zip1::LYS2 htz1::hphMX4 MIH1-GFP::kanMX6*	This work
DP1144	DP421 *htz1::URA3 spo11::ADE2*	This work
DP1153	DP421 *zip1::LYS2 cdc28-AF::LEU2*	This work
DP1154	DP421 *zip1::LYS2 htz1::hphMX4 cdc28-AF::LEU2*	This work

DP1157	DP421 *zip1::LYS2 swe1::LEU2*	This work
DP1174	DP421 *swr1::hphMX4*	This work
DP1185	DP421 *TPR1-P_{GAL1}-ZIP1-GFP ura3::P_{GPD1}-GAL4(848)ER::URA3/ura3-1*	This work
DP1186	DP421 *TPR1-P_{GAL1}-ZIP1-GFP P_{GPD1}-GAL4(848)ER::URA3/ura3-1 htz1::hphMX4*	This work
DP1259	DP421 *htz1::URA3 mek1::kanMX6*	This work
DP1353	DP421 *3MYC-SWE1*	This work
DP1354	DP421 *zip1::LYS2 3MYC-SWE1*	This work
DP1359	DP421 *TUB1/TUB1-GFP::TRP1*	PSS Lab
DP1360	DP421 *zip1::LYS2 TUB1/TUB1-GFP::TRP1*	PSS Lab
DP1364	DP421 *zip1::LYS2 rad51::natMX4 TUB1/TUB1-GFP::TRP1*	PSS Lab
DP1414	DP421 *zip1::LYS2 htz1::hphMX4 3MYC-SWE1*	This work
DP1416	DP421 *zip1::LYS2 htz1::hphMX4 cdc28-AF::LEU2 3MYC-SWE1*	This work
DP1441	DP421 *zip1::LYS2 htz1::URA3 sum1::natMX4*	This work
DP1445	DP421 *[hta1-htb1]::kanMX6 [hta2-htb2]::natMX4* pSS347 (*HTA1-HTB1*)-*TRP1*	This work
DP1446	DP421 *[hta1-htb1]::kanMX6 [hta2-htb2]::natMX4* pSS348 (*HTA1-htb1-Y40F*)-*TRP1*	This work
DP1449	DP421 *zip1::LYS2 htz1::hphMX4 [hta1-htb1]::kanMX6 [hta2-htb2]::natMX4* pSS347 (*HTA1-HTB1*)-*TRP1*	This work
DP1450	DP421 *zip1::LYS2 htz1::hphMX4 [hta1-htb1]::kanMX6 [hta2-htb2]::natMX4* pSS348 (*HTA1-htb1-Y40F*)-*TRP1*	This work
DP1467	DP421 *zip1::LYS2 3MYC-swe1-N584A*	This work
DP1468	DP421 *zip1::LYS2 htz1::hphMX4 3MYC-swe1-N584A*	This work
DP1523	DP421 *spo11::ADE2*	This work
DP1524	DP421 *zip1::LYS2 spo11::ADE2*	This work
DP1525	DP421 *zip1::LYS2 p306(BrdU-Inc)::URA3/ura3-1*	This work
DP1526	DP421 *zip1::LYS2 htz1::hphMX4 p306(BrdU-Inc)::URA3/ura3-1*	This work

* All strains are diploids isogenic to BR1919 (Rockmill and Roeder 1990), except strains DP969, DP973 and DP974, which are diploids isogenic to SK1. The haploid parents of DP969 (SK1) were obtained from S. Keeney (Thacker *et al.* 2011). Unless specified, all strains are homozygous for the indicated markers. DP421 is a *lys2* version of the original BR1919-2N.

Rockmill, B., and G. S. Roeder, 1990 Meiosis in asynaptic yeast. Genetics 126: 563-574.

Thacker, D., I. Lam, M. Knop and S. Keeney, 2011 Exploiting spore-autonomous fluorescent protein expression to quantify meiotic chromosome behaviors in *Saccharomyces cerevisiae*. Genetics 189: 423-439.

Table S2. Plasmids

Plasmid	Vector	Relevant parts	Source/Reference
pTK17	pUC19	*htz1::URA3*	(SANTISTEBAN *et al.* 2000)
pJC29	pRS426	*2μ URA3 CDC5*	(JASPERSEN *et al.* 1998)
pR2042	pRS305	*LEU2 cdc28-AF*	(LEU and ROEDER 1999)
pR2045	pRS426	*2μ URA3 CLB1*	(LEU and ROEDER 1999)
pSS263	pRS426	*2μ URA3 NDT80*	This work
pSS248	pYES2 derivative	*2μ URA3* P_{HOP1}*-GFP*	This work
pSS265	pSS248	*2μ URA3* P_{HOP1}*-GFP-MIH1*	This work
pSS345	pRS316	*CEN6 URA3 HTA1-HTB1*	This work
pSS347	pRS314	*CEN6 TRP1 HTA1-HTB1*	This work
pSS348	pRS314	*CEN6 TRP1 HTA1-htb1-Y40F*	This work
pSS354	pSS248	*2μ URA3* P_{HOP1}*-GFP-BDF1*	This work

JASPERSEN, S. L., J. F. CHARLES, R. L. TINKER-KULBERG and D. O. MORGAN, 1998 A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerevisiae*. Mol Biol Cell **9:** 2803-2817.

LEU, J. Y., and G. S. ROEDER, 1999 The pachytene checkpoint in *S. cerevisiae* depends on Swe1-mediated phosphorylation of the cyclin-dependent kinase Cdc28. Mol Cell **4:** 805-814.

SANTISTEBAN, M. S., T. KALASHNIKOVA and M. M. SMITH, 2000 Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. Cell **103:** 411-422.

Table S3. Primary antibodies

Antibody	Host and type	Application* (Dilution)	Source / Reference
Cdc2-Y15-P (Cdc28-Y19-P)	Rabbit polyclonal	WB (1:1000)	Cell Signaling Technology #9111
Phospho-(S/T)Q	Rabbit polyclonal	IF (1:200)	Cell Signaling Technology #2851
Cdc5	Goat polyclonal	WB (1:1000)	Santa Cruz Biotechnology sc-6733
Cdc28 (PSTAIRE)	Rabbit polyclonal	WB (1:1000)	Santa Cruz Biotechnology; sc53
Clb1	Goat polyclonal	WB (1:100)	Santa Cruz Biotechnology; sc-7647
Hed1	Rabbit polyclonal	WB (1:20000)	N. Hollingsworth (CALLENDER *et al.* 2016)
Hed1-T40-P	Rabbit polyclonal	WB (1:50000)	N. Hollingsworth (CALLENDER *et al.* 2016)
Hop1-T318-P	Rabbit polyclonal	WB (1:1000)	J. Carballo (PENEDOS *et al.* 2015)
H3-T11-P	Rabbit polyclonal	WB (1:2000)	Abcam ab5168
Mek1	Rabbit polyclonal	WB (1:1000)	PSS Lab (ONTOSO *et al.* 2013)
Ndt80	Rabbit polyclonal	WB (1:5000)	M. Lichten (BENJAMIN *et al.* 2003)
Rad51	Rabbit polyclonal	IF (1:300)	Santa Cruz Biotechnology sc-33626
Myc	Rabbit polyclonal	WB (1:1000)	Sigma c3956
Pch2	Rabbit polyclonal	IF (1:400)	PSS Lab / R. Freire
GFP (JL-8)	Mouse monoclonal	WB (1:2000-10000)	Clontech 632381
PGK (22C5D8)	Mouse monoclonal	WB (1:2000)	Invitrogene 459240
Tubulin (TAT1)	Mouse monoclonal	WB (1:10000) IF (1:500)	K. Gull (ACOSTA *et al.* 2011)

*WB, western blot; IF, immunofluorescence

ACOSTA, I., D. ONTOSO and P. A. SAN-SEGUNDO, 2011 The budding yeast polo-like kinase Cdc5 regulates the Ndt80 branch of the meiotic recombination checkpoint pathway. Mol Biol Cell **22:** 3478-3490.

BENJAMIN, K. R., C. ZHANG, K. M. SHOKAT and I. HERSKOWITZ, 2003 Control of landmark events in meiosis by the CDK Cdc28 and the meiosis-specific kinase Ime2. Genes Dev **17:** 1524-1539.

CALLENDER, T. L., R. LAUREAU, L. WAN, X. CHEN, R. SANDHU *et al.*, 2016 Mek1 Down Regulates Rad51 Activity during Yeast Meiosis by Phosphorylation of Hed1. PLoS Genet **12:** e1006226.

ONTOSO, D., I. ACOSTA, F. VAN LEEUWEN, R. FREIRE and P. A. SAN-SEGUNDO, 2013 Dot1-dependent histone H3K79 methylation promotes activation of the Mek1 meiotic checkpoint effector kinase by regulating the Hop1 adaptor. PLoS Genet **9:** e1003262.

PENEDOS, A., A. L. JOHNSON, E. STRONG, A. S. GOLDMAN, J. A. CARBALLO *et al.*, 2015 Essential and Checkpoint Functions of Budding Yeast ATM and ATR during Meiotic Prophase Are Facilitated by Differential Phosphorylation of a Meiotic Adaptor Protein, Hop1. PLoS One **10:** e0134297.