Supplemental Data
Control of Meiosis by Respiration

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Supplemental Experimental Procedures

Yeast Strains and Plasmids

All *Saccharomyces cerevisiae* strains are SK1 derivatives and are listed in Table S1; *S. pombe* strains in Table S2. *PET100* and *RIM101* disruptions, *IME1*-HA, and *MSN2*-GFP alleles were constructed by the method of Longtine et al. [S1]. A triple HA tag was introduced in-frame after codon 532 of *RIM101* by the method described by Schneider et al. [S2]. *GAL4.ER*, *GAL-NDT80*, and *ime2-as* alleles are described in Benjamin et al. [S3]. *spo11*Δ and *cdc6-mn* alleles are described in Hochwagen et al. [S4]. *Schizosaccharomyces pombe* strains PHP4, PHP14, PHP25, and TF205 are described in Haffter et al. [S5]. p15 and p16 were created by treating of PHP25 and TF205, respectively, with ethidium bromide as described by Haffter et al. [S5] and screened for complete loss of mitochondrial DNA by DAPI staining. The *IME1*-LacZ reporter (p1335) was constructed by the cloning of a fragment containing 4 kb upstream of the *IME1* start codon as well as amino acids 1–68 of the *IME1* ORF in-frame with LacZ in the *Eco*RI and *Sal*I sites of YIp355. The resulting plasmid was cut with *Eco*NI and integrated at the *IME1* promoter.

Yeast Media and Culture

For sporulation, cells were grown to saturation in YPD (OD_{600} of 9–10 for WT strains, or 7–8 for *pet100*Δ), diluted to an OD_{600} of 0.3 in YPA (1% yeast extract, 2% bactopeptone, 1% potassium acetate), and incubated for 16 hr where indicated; washed; and resuspended at an OD_{600} of 1.8 in 0.3% potassium acetate pH7 (KAc) or 0.22% potassium chloride (KCl) medium. For induction of *GAL-NDT80*, cells were incubated in the indicated medium for 6 hr and released from the arrest by addition of β-estradiol to a final concentration of 1 μM. For inhibition of *ime2-as*, cells were incubated in KAc medium for 4 hr in the presence of 20 μM 1-NA-PP1 [S3]. Cells were released by washing with at least 10 volumes of water and resuspended in the indicated medium. Oligomycin was used at a final concentration of 32 μg/ml, dinitrophenol (DNP) at 10 mM, rapamycin at 100 nM, and sodium azide at 5 mM. *S. pombe* strains were cultured on YES media, and mating/ sporulation was conducted on Malt Extract media.

4',6-diamidino-2-phenylindole (DAPI) Staining and Indirect In Situ Immunofluorescence

Cells were permeabilized in 70%–80% ethanol for at least ten minutes and resuspended in 1 μg/ml DAPI. At least 100 cells were counted for each sample. Costaining with DAPI and anti-tubulin antibodies was performed as described by Visintin et al. [S6]. At least 200 cells were counted for each sample.

Other Methods

For Western-blot analysis, samples were prepared as described in Moll et al. [S7]. Immunoblots were performed as described by Cohen-Fix et al. [S8], with HA.11 antibody at 1:1000 dilution and PGK1 antibody at 1:10,000. For Northern-blot analysis, total RNA was prepared as described in Corss et al. [S9], and Northern blotting was performed as described in Hochwagen et al. [S4]. Membranes were stained with methylene blue (0.3 M sodium acetate, 0.03% w/v methylene blue, [pH5.2]) to visualize total RNA. poly(A)+ RNA was isolated as described by Nautival et al. [S10] with the use of 700 μg total RNA prepared by acid phenol extraction [S11]. For cell-viability analysis, cells were incubated in the indicated medium at an OD_{600} of 1.8 and cell density was measured by coulter counter. Five hundred cells from each sample were spread in triplicate on YPD plates and incubated at 30°C. After 2 days, colony-forming units were counted. Ethanol content was measured in the supernatant of cell cultures with the use of a YSI 7100 MBS machine according to manufacturer's instructions. β-galactosidase assays were performed as described by Bernstein et al. [S12], except that Yeast Protein Extraction Reagent (Pierce) was used to lyse cells. DNA-content analysis was performed by flow cytometry as described by Visintin et al. [S13]. Msn2-GFP cells were fixed and DAPI stained as described by Monje-Casas et al. [S14].

References

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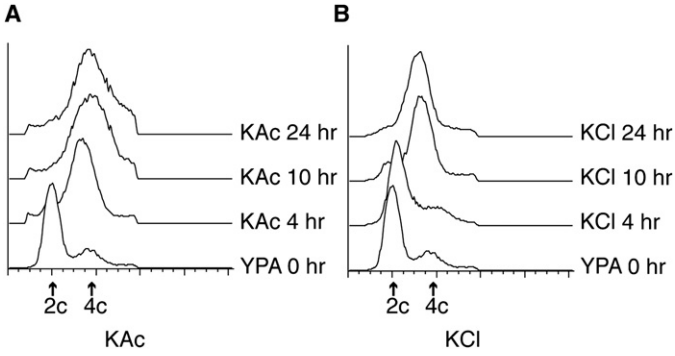

Figure S1. Premeiotic DNA Replication Occurs with a Delay in the Absence of a Carbon Source

DNA content of *ime2-as* cells (A12113) incubated in (A) KAc or (B) KCl without inhibitor.

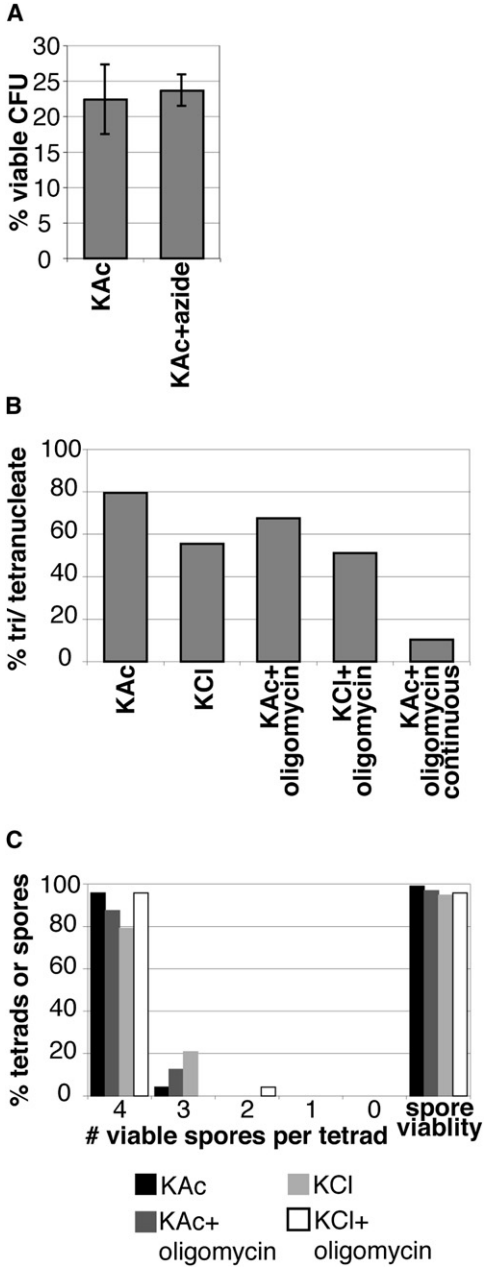

Figure S2. Effects of aziDe and Oligomycin on Cell Viability and Sporulation of *GAL-NDT80* Cells

(A) *GAL-NDT80* cells (A14201) were incubated in KAc for 6 hr. β-estradiol was added to induce *NDT80* in the presence or absence of 5 mM sodium azide. Five hundred cells from each sample were plated on YPD in triplicate 24 hr after the addition of azide, and the number of colony-forming units was determined after 2 days of incubation. Error bars represent standard deviation.

(B) The percentage of tri- and tetranucleate cells was determined in the *GAL-NDT80* strain (A14201) in the presence or absence of oligomycin after 24 hr by DAPI staining. Cells were incubated continuously in oligomycin before and after *NDT80* induction (KAc+oligomycin continuous) or incubated in KAc and then shifted to KAc or KCl with or without oligomycin at the time of *NDT80* induction as in Figure 1E.

(C) Distribution of tetrad classes and total spore viability of tetrads prepared from cells treated as described in (B). n = 24.

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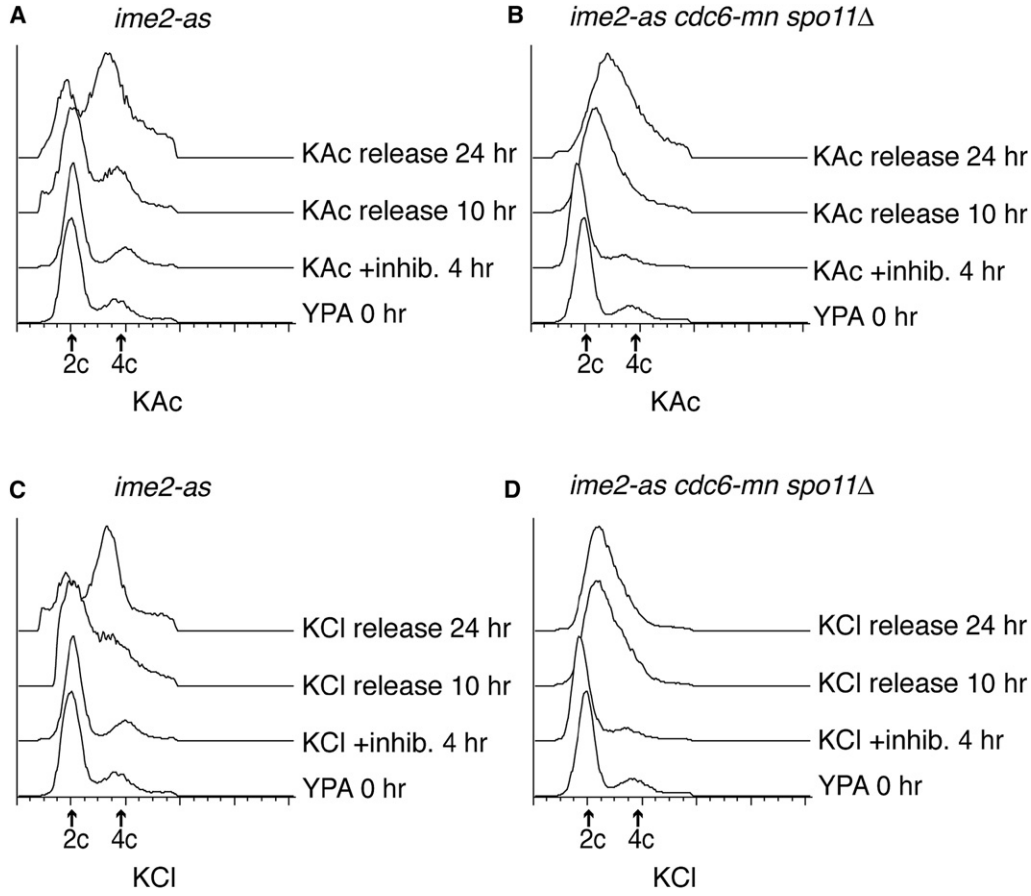

Figure S3. Premeiotic DNA Replication is Inhibited in Cells Lacking *CDC6*

DNA content of *ime2-as* (A12113) cells from Figure 1H (A, C) or *ime2-as cdc6-mn spo11*Δ (A17082) cells from Figure 1J (B, D) released into KAc (A, B) or into KCl (C, D) after 4 hr of Ime2 inhibition with 20 μM 1-NA-PP1 in KAc.

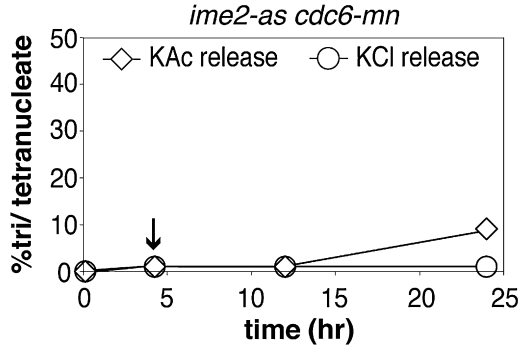

Figure S4. Eliminating Premeiotic DNA Replication Does Not Bypass the Need for a Carbon Source prior to Metaphase I

The percentage of tri- and tetranucleate cells was determined in the *ime2-as cdc6-mn* strain (A16701) at the indicated times by DAPI staining. Cells were released into KAc or KCl after 4 hr of Ime2 inhibition with 20 μM 1-NA-PP1 in KAc. The arrow indicates the time at which the inhibitor was washed out. Three independent replicates of this experiment were conducted; results shown are typical of all replicates.

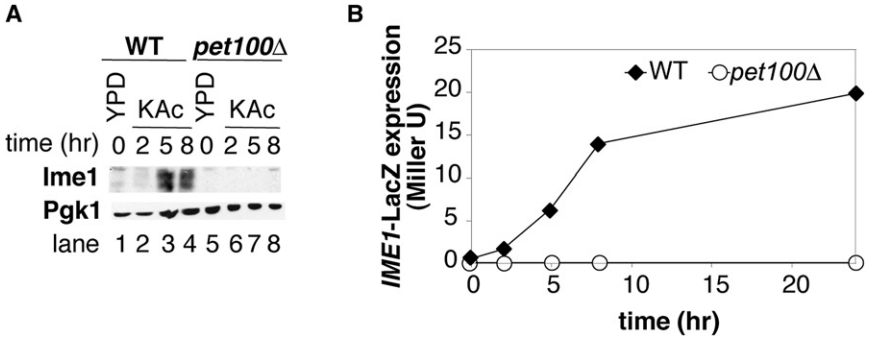

Figure S5. *IME1* Does Not Express in Petite Cells

(A) Ime1-3HA protein levels were determined in stationary phase WT (A19413) or *pet100*Δ (A19414) cells pregrown in YPD (lanes 1, 5) and at the indicated times after shift to KAc (lanes 2–4, 6–8). Pgk1 is shown as a loading control.

(B) *IME1*-LacZ activity of WT or *pet100*Δ cells from (A).

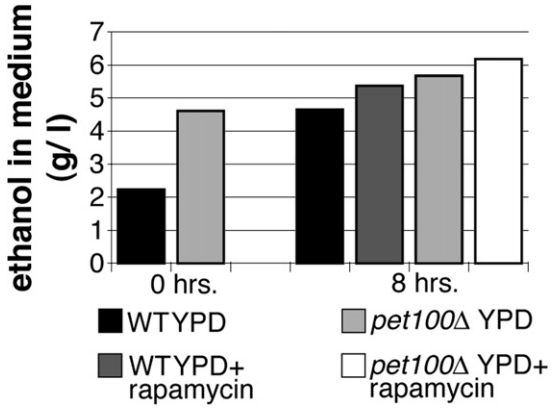

Figure S6. Glycolysis is Not Impaired in the Presence of Rapamycin

An independent set of WT (A15869) or *pet100*Δ (A15871) cells was treated with or without rapamycin as in Figure 2, and ethanol content of the supernatant was measured at the time of or 8 hr after rapamycin addition.

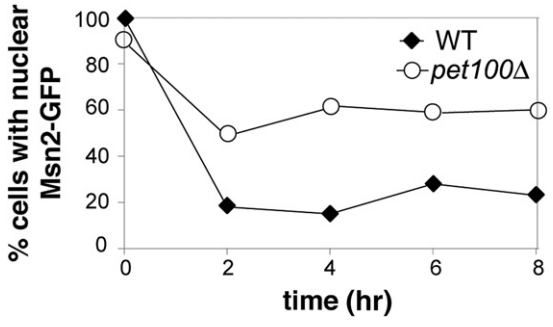

Figure S7. Msn2 Expression or Localization is Not Defective in Petite Cells

Percent of WT (A16902) or *pet100*Δ (A16903) cells with nuclear Msn2-GFP signal at the indicated time points after growth to stationary phase in YPD (time 0) or after transfer to sporulation medium.

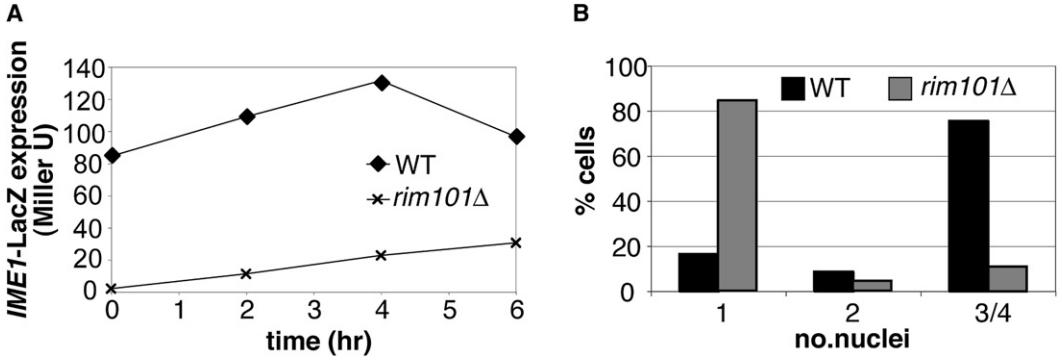

Figure S8. Deletion of *RIM101* Decreases *IME1* Expression and Sporulation

(A) *IME1*-LacZ activity was measured in WT (A15869) or *rim101*Δ (A18590) cells in YPA cultures (0 hr time point) or after incubation in KAc for the indicated times.

(B) Nuclear divisions of cells shown in (A) were measured by DAPI staining after 24 hr.

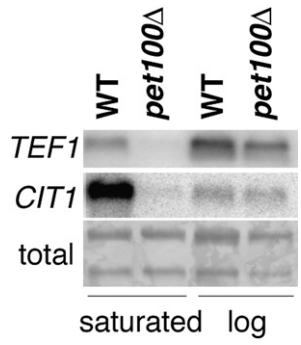

Figure S9. *TEF1* and *CIT1* Expression in the Presence or Absence of Functional Mitochondria

TEF1 and *CIT1* transcripts were detected by Northern blotting in WT or *pet100*Δ cells in saturated or logarithmic phase in YPD medium. Total RNA is shown as a loading control.

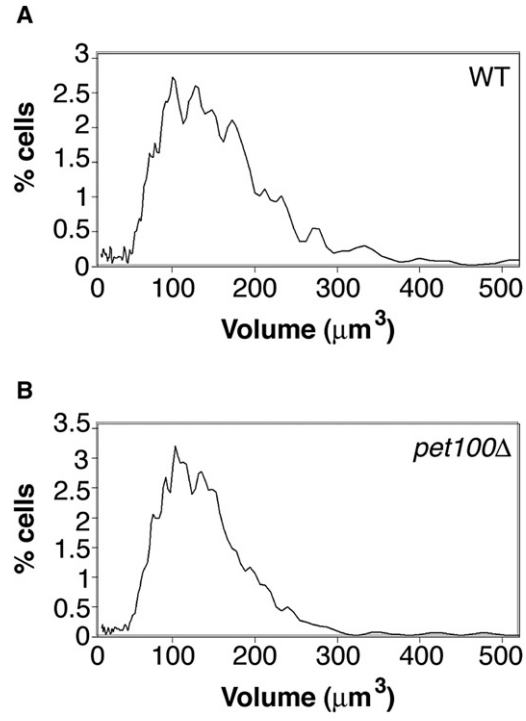

Figure S10. WT and Petite Cells Display Similar Cell Volumes

Cell volumes were analyzed of (A) WT (A15869) or (B) *pet100*Δ (A15871) strains shown in Figure 2 at the 0 hr time point.

Table S1. *S. cerevisiae* Strains Used in This Study

Strain	Genotype
A15869	*MAT*a/alpha, *IME1*::p*IME1*-LacZ-*URA3, RIM101*-3xHA/*RIM101, ho*::*LYS2*, *lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A15871	*MAT*a/alpha, *pet100Δ::*KanMX, *IME1*::p*IME1*-LacZ-*URA3, RIM101*-3xHA/*RIM101, ho*::*LYS2*, *lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A12113	*MAT*a/alpha, *ime2-as1*-myc::*TRP1*(M146G), *ho*::hisG, *lys2, ura3, leu2*::hisG, *trp1*::hisGDGA, *his3-11*
A14201	*MAT*a/ alpha, *GAL-NDT80*::*TRP1*, *ura3*::p*GPD1-GAL4*(848).ER::*URA3*, *ho*::*LYS2*, *lys2*, *LEU2*::hisG, *his3*::hisG, *trp1*::hisG
A16701	*MAT*a/alpha p*SCC1*-3xHA-*CDC6*-KanMX, *ime2-as1*-myc::*TRP1*(M146G), *ho*::*LYS2*, *his3*, *leu2*, *ura3*, *trp1*, *lys2*
A16902	*MAT*a/alpha, *IME1*::p*IME1*-LacZ-*URA3*/*IME1*, *MSN2*-GFP-KanMX, *ho*::*LYS2*, *lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A16903	*MAT*a/alpha, *pet100Δ::*KanMX, *IME1*::p*IME1*-LacZ-*URA3*/*IME1*, *MSN2*-GFP-KanMX, *ho*::*LYS2*, *lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A17082	*MAT*a/alpha p*SCC1*-3xHA-*CDC6*-KanMX, *spo11*Δ::*URA3*, *ime2-as1*-myc::*TRP1*(M146G), *ho*::*LYS2*, *his3*, *leu2*, *ura3*, *trp1*, *lys2*
A17090	*MAT*a/alpha, *IME1*::p*IME1*-LacZ-*HIS3*, *ho*::*LYS2, lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A17091	*MAT*a/alpha, *pet100Δ::*KanMX, *IME1*::p*IME1*-LacZ-*HIS3*, *ho*::*LYS2, lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A18415	*MAT*a/alpha *spo11*Δ::*URA3*, *ime2-as1*-myc::*TRP1*(M146G), *ho*::*LYS2*, *his3*, *leu2*, *ura3*, *trp1*, *lys2*
A18590	*MAT*a/alpha, *rim101Δ::*KanMX, *IME1*::p*IME1*-LacZ-*URA3, RIM101*-3xHA/*RIM101, ho*::*LYS2*, *lys2*, *ura3*, *leu2*::hisG, *his3*::hisG, *trp1*::hisG
A19413	*MAT*a/ alpha, *IME1*-HA3::KanMX6/ *IME1*::p*IME1*-LacZ-*URA3, ho*::*LYS2*, *leu2*::hisG, *HIS3*/ *his3*::hisG, *ura3*, *lys2*, *trp1*::hisG
A19414	*MAT*a/ alpha, *pet100Δ::*KanMX, *IME1*-HA3::KanMX6/ *IME1*::p*IME1*-LacZ-*URA3, ho*::*LYS2*, *leu2*::hisG, *HIS3*/ *his3*::hisG, *ura3*, *lys2*, *trp1*::hisG

Table S2. *S. pombe* Strains Used in This Study

Strain	Genotype
p1	*Mat h-, ura4-D18, leu1-32, his3-D1, ade6-216*
p3	*Mat h-, ura4-D18, leu1-32, his3-D1, ade6-210*
p11 (PHP25)	*MAT h+, ura1-161, ade6-216, ptp1-1*
p12 (TF205)	*MAT h+, mat1-P, mat2,3::LEU2, leu1-32, ade6-M210, ptp1-1*
p13 (PHP4)	*MAT h-, ade6M-216, leu1-32, ptp2-1,* ρ0
p14 (PHP14)	*MAT h-, ade6M-216, leu1-32, ptp1-1,* ρ0
p15	*MAT h+, ura1-161, ade6-216, ptp1-1*, ρ0 (PHP25 derivative)
p16	*MAT h+, mat1-P, mat2,3::LEU2, leu1-32, ade6-M210, ptp1-1*, ρ0 (TF205 derivative)