SUPPLEMENTARY INFORMATIONS

METHODS

Yeast strains and plasmids. Deletions of the *MEC1*, *SML1*, *TEL1*, *MRE11* and *SAE2* genes have been obtained as previously described (Baroni *et al*, 2004; Clerici *et al*, 2004). All the strains carrying the deletion of *MEC1* are kept viable by deleting also the *SML1* gene. The *sml1Δ* strains were undistinguishable from wild type for the kinetics of DSB resection, cell cycle progression and Rad53 phosphorylation. To construct *EXO1* chromosomal deletion, the *exo1Δ::LEU2* cassette was obtained by PCR using plasmid pKKLEU2HR (kindly provided by K. Nasmyth, Vienna, Austria) as a template and oligonucleotides PRP592 (3'-ACT GAA AGG CGT AGA AAG GAA TGG GTA TCC AAG GTC TTC TTC CTC ATG CAG GCT AAC CGG AAC CTG-5') and PRP593 (3'-GTG CAG TAC TTA ACT TTT ATT TAC CTT TAT AAA CAA ATT GGG AAA CAT TGG CCC TAC CCA CAT ATG-5') as primers. One-step replacement of 1693 bp of the *EXO1* coding region with the *LEU2* cassette was carried out by transforming strain JKM139 and the isogenic *sae2Δ* and *GAL-SAE2* strains. Strains expressing fully functional Mre11 and Ddc2 tagged versions were constructed as previously described (Paciotti *et al*, 2000; Clerici *et al*, 2004). To generate the JKM139 strain carrying the *sae2*^{2,5,6,8,9} allele (alanine substitutions at S73, T90, S249, T279, S289) integrated at the *SAE2* locus, the JKM139 *sae2Δ* strain was transformed with *Apa*I-digested plasmid pML488 constructed as previously described (Baroni *et al*, 2004). To generate a *rad50s* mutant (*rad50-K81I*), JKM139 cells were transformed with *Msc*I-digested plasmid pML533, carrying the first 666 bp of *RAD50* gene with the K81I mutation (Clerici *et al*, 2005). To generate strains with three copies of the *GAL-SAE2* or the *GAL-sae2*^{2,5,6,8,9} fusions at the *URA3* locus, strains W303 and JKM139 were transformed with *Apa*I-digested plasmids pML508 and pML518, carrying fusions to the *GAL1* promoter of the entire *SAE2* or *sae2*^{2,5,6,8,9} coding regions, respectively. The JKM139 derivative *SAE2-HA*, *GAL-SAE2-HA* and *GAL-sae2*^{2,5,6,8,9}–HA strains, which carry, respectively, the fully functional *SAE2-HA* allele at the *SAE2* chromosomal locus, and the *GAL-SAE2-HA* or the *GAL-sae2*^{2,5,6,8,9}-HA fusions at the *URA3* chromosomal locus, were constructed by one step tagging as previously described (Baroni *et al*, 2004). Integration accuracy was verified by Southern blot analysis.

To obtain *cdc5-ad* mutants, the *Bst*EII-digested plasmid pRS306cdc5-ad, kindly provided by D.P. Toczyski, was used to transform JKM139 strain. Strains carrying the *cdc5-ad* allele at the *CDC5* locus were identified by screening for clones able to grow on 5-fluoroorotic acid. The presence of the *cdc5-ad* mutation was assessed by PCR and restriction fragment analysis. JKM139 *GAL-SAE2 cdc5-ad* strain was a meiotic segregant from a cross between strains JKM139 *MATα cdc5-ad* and JKM139 *MATa GAL-SAE2*. Strains JKM139 *mre11-D56N* and JKM139 *mre11-H125N* were meiotic segregants from crosses of strain JKM139 *MATα* with strains LSY1032 and LSY1397, respectively, kindly provided by L. Symington (Columbia University, New York, USA) (Moreau *et al*, 1999).

Standard yeast genetic techniques and media were according to Rose *et al*, 1990. Cells were grown in YEP medium (1% yeast extract, 2% bactopeptone, 50 mg/l adenine) supplemented with 2% glucose (YEPD) or 2% raffinose (YEP+raf) or 2% raffinose and 2% galactose (YEP+raf+gal).

To give rise to plasmid pML508, a fragment spanning from position +1 to position +1162 from the *SAE2* translation initiation codon was amplified by PCR using yeast genomic DNA as a template and oligonucleotides PRP559 (5'-CGC GGA TCC ATA TGG TGA CTG GTG AAG AAA ATG-3') and PRP560 (5'-AAC TGC AGC TGG TAA GTT AGG TGT CAT TTG-3') as primers, and was then cloned, together with the 700-bp *Eco*RI-*Bam*H1 fragment containing the *GAL1* promoter, into the *Eco*RI-*Pst*I sites of plasmid YIplac211 (Gietz & Sugino, 1988). To generate plasmid pML518, a fragment spanning from position +1 to position +1162 from the *SAE2* translation initiation codon was amplified by PCR using oligonucleotides PRP559 and PRP560 as primers and plasmid pML488 as a template (Baroni *et al*, 2004), and was then cloned, together with the 700-bp *Eco*RI-*Bam*H1 fragment containing the *GAL1* promoter, into the *Eco*RI-*Pst*I sites of plasmid YIplac211. To give rise to plasmid pML533, a 1051 bp fragment containing the promoter and the first 666 bp of the coding region of the *rad50s* K81I allele was amplified by PCR using oligonucleotides PRP706 (5'-CGG AAT TCC CGA TAG TAC TTC CAC TTA CAA TAC-3') and PRP707 (5'-CGG AAT TCC ATT GCT TTC GAT CTG TCT TTA TCC-3') as primers and yeast genomic DNA from MJL1699 strain (kindly provided by N. Kleckner, Harvard University, Cambridge, USA) as a template, and was then cloned into the *Eco*RI site of plasmid YIplac128

(Gietz & Sugino, 1988). The presence of the mutation was verified by nucleotide sequence analysis.

Other techniques. Flow cytometric DNA analysis was determined on a Becton-Dickinson FACScan. Nuclear division was scored with a fluorescence microscope on cells stained with propidium iodide. DSB end resection at the *MAT* locus was analyzed on alkaline agarose gels as described in Shroff *et al*, 2004, using a single-stranded probe complementary to the unresected DSB strand. This probe was obtained by *in vitro* transcription using Promega Riboprobe System-T7 and plasmid pML516 as a template. Plasmid pML516 was constructed by inserting in the pGEM-7Zf *Eco*RI site a 900-bp fragment containing part of the *MAT*α locus, obtained by PCR using yeast genomic DNA as a template and PRP643 (5'-CGG AAT TCC CTG GTT TTG GTT TTG TAG AGT GG-3') and PRP644 (5'-CGG AAT TCG AAA CAC CAA GGG AGA GAA GAC-3') as primers.

Immunofluorescence was performed as described in Fraschini *et al*, 1999. Immunostaining of Mre11-Myc18 was detected by incubation with 9E10 antibody followed by indirect immunofluorescence using CY3-conjugated goat anti-mouse antibody (Amersham Biosciences).

For western blot analysis, protein extracts were prepared by TCA precipitation as previously described (Clerici *et al*, 2004). Rad53 was detected using anti-Rad53 polyclonal antibodies kindly provided by J. Diffley (Clare Hall Laboratories, South Mimms, United Kingdom). Secondary antibodies were purchased from Amersham and proteins were visualized by an enhanced chemiluminescence system according to the manufacturer.

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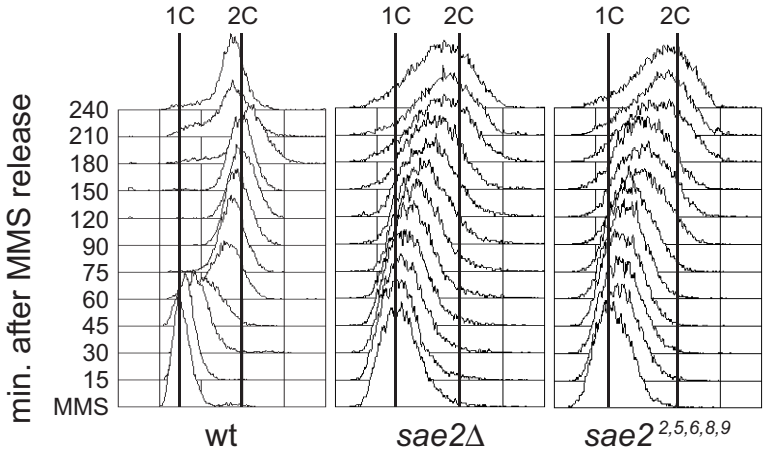
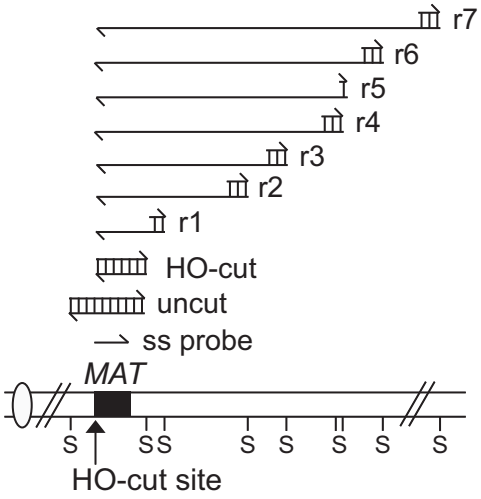
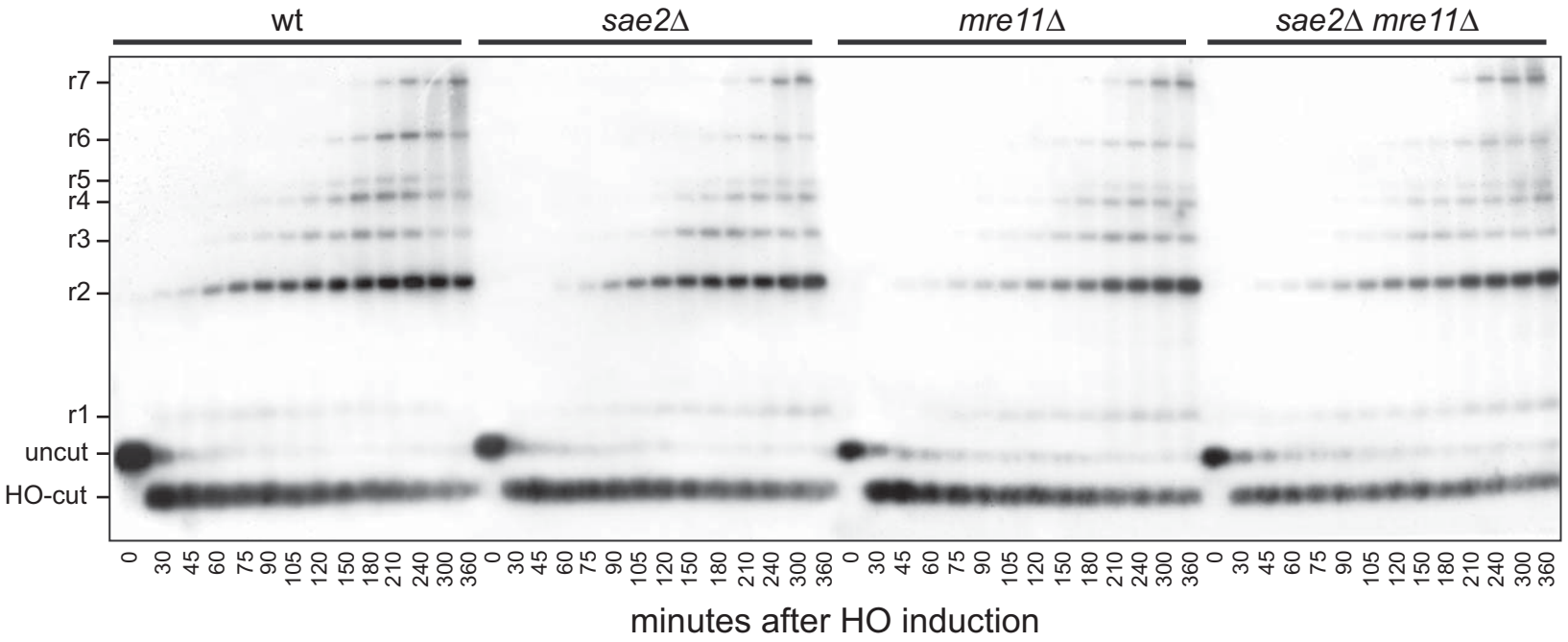
Supplementary Fig S1



Supplementary Fig S2



Supplementary Fig S3



LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Fig S1. Checkpoint response to MMS treatment in *sae2Δ* and *sae2*^{2,5,6,8,9} cells. Aliquots of the MMS-treated cultures in Fig. 1C were taken at the indicated time points after release from α-factor and MMS, and DNA content was measured by FACS analysis.

Supplementary Fig S2. Schematic representation of the method used to detect DSB formation and 5'-to-3' resection in the region immediately centromere-distal to the *MAT* HO site in JKM139 derivative strains. Genomic DNA digested with *Ssp*I and separated on alkaline agarose gel, followed by gel blot hybridization with a single-stranded RNA probe specific for the *MAT* locus, reveals HO-cut and uncut fragments of 0.9 kb and 1.1 kb, respectively. 5'-to-3' resection progressively eliminates *Ssp*I sites, that are located 1.7, 3.5, 4.7, 5.9, 6.5, 8.9 and 15.8 kb centromere-distal from the HO-cut site, producing larger *Ssp*I fragments (labeled r1 through r7) detected by the probe.

Supplementary Fig S3. The *MRE11* and *SAE2* genes belong to the same epistasis group with respect to DSB resection. YEP+raf nocodazole-arrested cell cultures of wild type JKM139 and isogenic *sae2Δ*, *mre11Δ* and *sae2Δ mre11Δ* strains were transferred to YEP+raf+gal in the presence of nocodazole at time zero. Genomic DNA prepared from samples withdrawn at the indicated times was digested with *Ssp*I, separated on alkaline agarose gel, and analyzed by Southern using a single-stranded RNA probe as described in Fig. S2.