Escape from Mitotic Arrest: An Unexpected Connection Between Microtubule Dynamics and Epigenetic Regulation of Centromeric Chromatin in *Schizosaccharomyces pombe*

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ABSTRACT Accurate chromosome segregation is necessary to ensure genomic integrity. Segregation depends on the proper functioning of the centromere, kinetochore, and mitotic spindle microtubules and is monitored by the spindle assembly checkpoint (SAC). In the fission yeast *Schizosaccharomyces pombe*, defects in Dis1, a microtubule-associated protein that influences microtubule dynamics, lead to mitotic arrest as a result of an active SAC and consequent failure to grow at low temperature. In a mutant *dis1* background (*dis1-288*), loss of function of Msc1, a fission yeast homolog of the KDM5 family of proteins, suppresses the growth defect and promotes normal mitosis. Genetic analysis implicates a histone deacetylase (HDAC)-linked pathway in suppression because HDAC mutants *clr6-1*, *clr3Δ*, and *sir2Δ*, though not *hos2Δ*, also promote normal mitosis in the *dis1-288* mutant. Suppression of the *dis* phenotype through loss of *msc1* function requires the spindle checkpoint protein Mad2 and is limited by the presence of the heterochromatin-associated HP1 protein homolog Swi6. We speculate that alterations in histone acetylation promote a centromeric chromatin environment that compensates for compromised *dis1* function by allowing for successful kinetochore-microtubule interactions that can satisfy the SAC. In cells arrested in mitosis by mutation of *dis1*, loss of function of epigenetic determinants such as Msc1 or specific HDACs can promote cell survival. Because the KDM5 family of proteins has been implicated in human cancers, an appreciation of the potential role of this family of proteins in chromosome segregation is warranted.

KEYWORDS Msc1; lysine demethylase KDM5; histone deacetylase (HDAC); Rb; PLU-1; RBP2; trichostatin; thiabendazole; Mad2; Dis1 (XMAP215, TOG1); Swi6 (HP1 homolog)

ACCURATE chromosome segregation relies on precise assembly of the multiprotein kinetochore complex, which mediates the link between chromosomes and the mitotic spindle (Przewłoka and Glover 2009). The histone H3 variant CENP-A, along with epigenetic marks on canonical histones, defines centromeric chromatin, which forms the template for kinetochore assembly (Allshire and Karpen 2008; Verdaasdonk and Bloom 2011). Kinetochores serve as the site

of spindle microtubule attachment (Santaguida and Musacchio 2009) and, along with the centromere, translate microtubule attachment status to mediate force balance, tension sensing, and spindle checkpoint control (Bloom 2014). When associations between chromosomes and the spindle are incorrect, the spindle assembly checkpoint (SAC) delays progression through mitosis by preventing the onset of anaphase (Musacchio and Salmon 2007; Foley and Kapoor 2013).

Fission yeast centromeric chromatin (Allshire and Ekwall 2015) consists of a central core (cnt) and internal inverted repeats (imr), defined by the presence of CENP-A and methylated histone H3 K4 chromatin, flanked by heterochromatic outer repeats (otr) marked by methylated histone H3 K9 (Partridge *et al.* 2000; Takahashi *et al.* 2000; Kniola *et al.* 2001). The constitutive centromere-associated network (CCAN) forms the inner kinetochore and consists of more than a dozen conserved

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proteins, including fission yeast Mis6 (CENP-I in mammalian cells) that promotes incorporation of CENP-A at the centromere (McAinsh and Meraldi 2011; Westhorpe and Straight 2015). The KMN network is composed of the tethering protein Knl1, the Mis12 complex, and the NDC80 complex, a heterotetramer consisting of Ndc80, Nuf2, Spc24, and Spc25, that links microtubules to the kinetochore (Petrovic *et al.* 2014). The Ndc80 protein has a loop domain that associates with the evolutionarily conserved microtubule-associated protein (MAP) Dis1; mutations in Ndc80 that eliminate Dis1 binding mimic loss of Dis1 function (Hsu and Toda 2011). As a member of the XMAP215/TOG1 family of proteins that modulate microtubule dynamics (Ohkura *et al.* 2001; Brouhard *et al.* 2008), Dis1 may serve to modify the behavior of spindle microtubules and/or the Ndc80 complex to stabilize kinetochore-bound microtubules (Cheerambathur and Desai 2014).

The XMAP215/TOG family of proteins mediates microtubule polymer assembly, spindle formation, kinetochore function, and cell morphogenesis (Slep 2010; Al-Bassam and Chang 2011). The gene encoding Dis1 was first identified in fission yeast in a cytologic screen of cold-sensitive (*cs*) mutants: while *dis1* is not essential for cell division at typical growth temperatures, loss of *dis1* function due to deletion (*dis1*Δ) or a point mutation (*dis1-288*) confers cold sensitivity (Ohkura *et al.* 1988; Nabeshima *et al.* 1995). At low temperature, *dis1* mutants exhibit the *dis* (defect in sister-chromatid disjoining) phenotype: mitotic arrest with hypercondensed chromosomes that fail to separate. The association of Dis1 with Ndc80 (Hsu and Toda 2011) suggests a model whereby Dis1 localization to kinetochores allows it to influence the polymerization of kinetochore microtubules. The *alp14* gene encodes a second XMAP215/TOG family protein in fission yeast (Garcia *et al.* 2001). While mutation or disruption of *dis1* results in cold sensitivity (Ohkura *et al.* 1988), disruption of *alp14* results in temperature sensitivity, and deletion of both leads to nonviability at temperatures permissive for each of the single mutants (Garcia *et al.* 2001).

We have reported previously that deletion of the gene encoding fission yeast Msc1 restores viability to cells lacking *dis1* function (Qiu *et al.* 2010). Msc1 is required for chromosome stability and was first isolated as a multicopy suppressor of loss of Chk1 function (Ahmed *et al.* 2004), a conserved protein kinase important for the DNA damage-induced cell-cycle checkpoint response (Bartek and Lukas 2003). Msc1 influences centromere-kinetochore function because it exhibits genetic interactions with the centromere-specific histone H3 variant CENP-A (encoded by *cnp1*), and the kinetochore components Mis6, a component of the CCAN, and Mis12, a component of the KMN network (Ahmed *et al.* 2007). Msc1 is a stoichiometric component of the Swr1 complex (Buchanan *et al.* 2009; Kim *et al.* 2009; Zofall *et al.* 2009; Hou *et al.* 2010). Msc1 shares with members of the mammalian KDM5 family of proteins a similar organization of multiple conserved domains including JmjN and JmjC domains, three PHD fingers, and a C₂C₅ zinc finger (Ahmed

et al. 2004; Blair *et al.* 2011). The PHD fingers of Msc1 possess E3 ubiquitin ligase activity (Dul and Walworth 2007). Cells with a deletion of the *msc1* gene (*msc1*Δ) exhibit increased levels of bulk histone H3 K9 and/or K14 acetylation (Ahmed *et al.* 2004). Full-length Msc1 or a C-terminal fragment of Msc1 containing two PHD fingers can coprecipitate HDAC activity (Ahmed *et al.* 2004).

Epigenetic aberrations are associated with several disease states, including cancer, and inhibitors of enzymes that alter epigenetic modifications, particularly HDAC inhibitors, are emerging to have clinical importance (Falkenberg and Johnstone 2014). HDACs are highly conserved across species and form multiprotein complexes. *Schizosaccharomyces pombe* has at least one representative member of each of the three phylogenetic classes of HDACs: Clr6 and Hos2 belong to class I, Clr3 belongs to class II, and Sir2 is a member of class III (Ekwall 2005).

In this study, we further investigate our reported observation that deletion of *msc1* alleviates the *cs* lethality associated with *dis1-288* (Qiu *et al.* 2010). Because Msc1 coprecipitates a histone deacetylase activity (Ahmed *et al.* 2004), we hypothesized that loss of HDAC activity, like loss of *msc1*, might similarly suppress the *dis1-288 cs* phenotype. Indeed, we found that survival of *dis1-288* is made possible by loss of function of specific HDAC proteins, namely, *clr6-1*, *clr3*Δ, and *sir2*Δ, though not by deletion of *hos2*. Improved viability correlates with alleviation of the *dis* phenotype and requires Mad2, a component of the SAC (He *et al.* 1997). Furthermore, the heterochromatin-associated protein Swi6 limits *msc1*Δ-mediated suppression of the *dis1-288* mutant; *i.e.*, simultaneous deletion of *swi6* and *msc1* in the context of *dis1-288* completely restores viability at low temperature and eliminates the *dis* phenotype, though deletion of *swi6* alone does little to restore viability to *dis1-288*. Our data lead us to suggest that in cells compromised for microtubule function by virtue of the *dis1-288* mutation, an altered epigenetic state at the centromere resulting from loss of function of Msc1 or select HDAC activities may permit silencing of the SAC, thereby allowing sister-chromatid segregation. Thus, by contributing to the maintenance of epigenetic marks at the centromere, Msc1 can influence the success of centromere-kinetochore-spindle interactions.

Materials and Methods

Strains and growth conditions

The genotypes for the *S. pombe* strains used in this study are listed in Supporting Information, Table S1. Standard fission yeast methods were used for cell culture and mating (Moreno *et al.* 1991). For spotting assays, cells were grown overnight to mid-log phase at appropriate permissive temperatures: *dis1-288* mutant strains (33°), *clr6-1* (25°), and *clr6-1 dis1-288* (28°). Fivefold serial dilutions were made and spotted on YES plates with the following components per liter: 5 g yeast extract; 30 g glucose; 150 mg each of adenine, uracil, leucine,

lysine, and histidine; and 20 g agar. Then the plates were incubated at a range of temperatures for 5 days.

For trichostatin A (TSA; Sigma) sensitivity assays, serially diluted cells were spotted either on YES plates or on YES plates containing 5 μ g/ml TSA and incubated at 30°. For thiabendazole (TBZ; Sigma) sensitivity assays, serially diluted cells were spotted on YES plates or on YES plates containing 10 μ g/ml TBZ and incubated at 25 or 32°.

Microscopy

Microscopy studies were performed with cells grown overnight to mid-log phase at appropriate permissive temperatures. Cells were fixed in 0.5% glutaraldehyde and resuspended in water. For DAPI staining, 2 μ l of cells was mixed with 2 μ l of 10 μ g/ml DAPI (4',6-diamidino-2-phenylindole) solution. The cell suspension was analyzed using a fluorescence microscope (Zeiss Axioplan 2). Images were captured with a Zeiss AxioCam and analyzed with Openlab software.

For evaluating the *dis* phenotype, at least 150 dividing cells were counted for each sample. For cell length measurements, at least 30 septated cells were measured using the ruler tool in Openlab software.

Data availability

Strains are available on request.

Results

Loss of function of *Msc1* or specific HDACs promotes normal segregation in mutant cells exhibiting the *dis* phenotype

Deletion of *msc1* or *swr1* alone or both together results in suppression of the *cs* lethality of *dis1-288* mutant cells (Qiu *et al.* 2010). To determine whether deletion of *msc1* can suppress a null allele of *dis1* or the other fission yeast XMAP215/TOG homolog *alp14*, double mutants were constructed with *dis1 Δ* and *alp14 Δ* . Like *dis1-288*, *dis1 Δ* exhibits cold sensitivity (Nabeshima *et al.* 1995), while *alp14 Δ* exhibits temperature sensitivity (Garcia *et al.* 2001). As shown in Figure S1, deletion of *msc1* suppresses the conditional lethality of the *dis1 Δ* strain, while it compromises growth of the *alp14 Δ* *ts* mutant even below its restrictive temperature.

In light of our previous observation that a C-terminal fragment of *Msc1* can recruit HDAC activity, we questioned whether an altered epigenetic state resulting from deletion of *msc1* might be the mechanism for suppression of the *dis1* cold sensitivity. To test this, we formulated the hypothesis that in the context of the *dis1-288* mutant, loss of HDAC activity may mimic loss of *msc1*. Deletion mutants of HDAC-encoding genes *clr3*, *sir2*, and *hos2* are viable, whereas the HDAC encoded by *clr6* is an essential gene requiring use of a *ts* point mutant *clr6-1* to confer loss of *Clr6* function (Grewal *et al.* 1998). Double mutants of *dis1-288* with deletion or point mutants of the HDAC-encoding genes were constructed and

tested for growth at the restrictive temperature for *dis1-288*. As shown in Figure 1A, in comparison to a wild-type strain, the *dis1-288* mutant strain exhibits cold sensitivity at 22°. The double mutant *msc1 Δ* *dis1-288* is able to suppress the *dis1-288* growth defect, as reported previously (Qiu *et al.* 2010). Likewise, loss of HDAC activity resulting from mutation of *clr6-1* suppresses the *dis1-288* *cs* phenotype because the double mutant *clr6-1* *dis1-288* grows robustly at 22°. Deletion of *clr3* or *sir2* also suppresses the *dis1-288* *cs* phenotype at 22°, similar to *msc1 Δ* *dis1-288*. However, the double mutant *hos2 Δ* *dis1-288* fails to grow at 22°.

To determine whether viability of double-mutant *dis1-288* strains correlated with the *dis* phenotype, we examined cells by microscopy. Cells were grown overnight to mid-log phase at 33° for all strains except *clr6-1* *dis1-288*, which was grown at its permissive temperature of 28°. The next day, half the culture was fixed with glutaraldehyde, and the other half was shifted to 22° for 4 hr prior to fixation. Cells were stained with DAPI, observed under a fluorescence microscope, and photographed (Figure 1B). Dividing cells were classified on the basis of the chromosome segregation pattern: normal (both daughter cells contained equal intensity of DAPI staining representing three decondensed chromosomes, as observed in wild-type cells) or the *dis* phenotype. All three variations of the *dis* phenotype were evident, as illustrated schematically in Figure 1C: hypercondensed chromatin distributed evenly between two daughter cells (hypercondensed) or in a 2:1 or 3:0 ratio (Ohkura *et al.* 1988). As shown in Figure 1B and quantified in Figure 1C, wild-type cells exhibited normal mitosis with equal chromosome segregation at 33 and 22°. By contrast, the *dis* phenotype was evident at 22° in 80% of *dis1-288* cells (Figure 1, B and C). Deletion of the *msc1*, *clr3*, or *sir2* genes or mutation of *clr6-1* alleviates the *dis* phenotype to varying extents, with the most dramatic suppression seen in the *clr6-1* *dis1-288* strain. Only 20% of the population of *clr6-1* *dis1-288* double-mutant cells exhibited the *dis* phenotype, representing a fourfold increase in cells with normal chromosome segregation. The *msc1 Δ* *dis1-288* strain exhibited a threefold increase in cells with normal segregation, and both *clr3 Δ* *dis1-288* and *sir2 Δ* *dis1-288* double-mutant strains exhibited a 2.5-fold increase in the percentage of cells exhibiting normal segregation. However, the *hos2 Δ* *dis1-288* strain exhibited an increase in the *dis* phenotype by 8%. Thus, the improvement in colony formation observed in spotting assays (Figure 1A) as a consequence of eliminating *msc1* or HDAC function correlates with alleviating the *dis* phenotype (Figure 1, B and C).

The *dis1-288* mutant exhibited a slight cell-elongation phenotype (Ohkura *et al.* 1988). We detected a noticeable reduction in cell length in the *msc1 Δ* *dis1-288* double mutant (Figure 1D). For each strain, the mean length and SD of $n > 30$ dividing cells were calculated for cells grown at permissive temperature or shifted to 22° for 4 hr. As shown in Figure 1D, the mean cell lengths of *msc1 Δ* , *clr3 Δ* , *sir2 Δ* , and *hos2 Δ* mutants are in the range of that for wild-type cells, whereas the *dis1-288* mutant is slightly longer than wild-type cells at

Figure 1 In *dis1* mutant cells exhibiting mitotic arrest, loss of function of *msc1* or histone deacetylase activity promotes normal mitosis. (A) Cell growth on day 5 at 22° (restrictive temperature for *dis1-288*), 33° (permissive temperature for *dis1-288*), or 36° (restrictive temperature for *clr6-1*) of fivefold serial dilutions of the indicated strains. The single mutants *msc1Δ*, *clr3Δ*, *hos2Δ*, and *sir2Δ* are viable at all temperatures tested, while the *clr6-1* strain is temperature sensitive (Figure S1A). (B) Representative images of glutaraldehyde-fixed cells grown to mid-log phase at permissive temperature (33 or 28° as indicated) or shifted for 4 hr to the restrictive temperature for *dis1-288* (22°). Fixed cells were stained with DAPI and photographed under a fluorescence microscope. Asterisks indicate cells exhibiting normal mitosis. Arrows indicate various versions of *dis* phenotype: open-end arrow (2:1),

temperatures both permissive and restrictive for cell growth. Consistent with effects on the *dis* phenotype, deletion of *msc1*, *clr3*, or *sir2* reduced mean cell length in combination with the *dis1-288* mutant, whereas deletion of *hos2* exaggerated the elongated-cell phenotype of *dis1-288*. As reported previously, *clr6-1* cells are elongated (Hayles *et al.* 2013), as are *clr6-1 dis1-288* cells.

A functional SAC is necessary for *msc1Δ*-mediated suppression of *dis1-288 cs* phenotype

Deletion of *msc1* or reduction in HDAC activity promotes dissociation of sister chromatids despite loss of *dis1* function resulting in apparently normal mitosis and improved viability. Therefore, we hypothesized that a functional SAC may be necessary for the observed improvement in cell survival. Mad2 is a SAC protein that is recruited to unattached kinetochores at the beginning of mitosis and released when spindle attachment is complete, thereby triggering the loss of cohesin from sister chromatids and promoting the onset of anaphase (He *et al.* 1997; Saitoh *et al.* 2005; Musacchio and Salmon 2007). To test a requirement for Mad2 in allowing suppression of *dis1-288* by *msc1Δ*, we first attempted to make a *mad2Δ dis1-288* double mutant. When the cross between a *mad2Δ* strain and a *dis1-288* strain was performed at the typical temperature of 25°, we were unable to obtain any viable double mutants by random spore analysis. However, we found that we could isolate at low frequency a viable *mad2Δ dis1-288* strain by conducting the mating and sporulation at 36°, a temperature at which *dis1-288* is presumed to be fully functional (Figure S2).

With a viable strain of *mad2Δ dis1-288* in hand, we crossed it with an *msc1Δ dis1-288* strain to generate the triple mutant *msc1Δ mad2Δ dis1-288*. The cell growth phenotype of the triple mutant was determined by spotting assay at various temperatures in comparison with the corresponding single and double mutants (Figure 2A). Compared to *dis1-288* cells at 22 or 25°, the *mad2Δ dis1-288* strain exhibited reduced fitness. This suggests that an intact SAC is required when *Dis1* function is compromised. Further, combining *mad2Δ* with the double mutant *msc1Δ dis1-288* severely compromised the fitness of the triple mutant. Hence, suppression of the *dis1-288 cs* phenotype conferred by loss of function of *Msc1* requires Mad2 and a functional SAC.

To test whether the decreased viability observed in the preceding double- and triple-mutant strains correlated with the *dis* phenotype, we performed microscopy analysis as described for Figure 1 (B and C). Compared to the single *dis1-288* mutant, both the *mad2Δ dis1-288* and the *msc1Δ mad2Δ dis1-288* strains exhibited a 5–7% increase in the *dis* phenotype (Figure 2C). In the *mad2Δ dis1-288* and *msc1Δ mad2Δ dis1-288* mutants, a variation of the *dis* phenotype was observed that displayed decondensed chromosomes and the “cut” phenotype (exiting mitosis in the absence of chromo-

some segregation). Strikingly, in comparison to the *msc1Δ dis1-288* strain, for which 65% of cells examined exhibit a normal mitosis, the introduction of *mad2Δ* reduced the percentage of cells exhibiting normal mitosis by fourfold. Thus, the normal mitosis observed in *msc1Δ dis1-288* cells clearly depends on Mad2 and a functional SAC. Despite the compromised viability of cells lacking *mad2*, cell length remained similar to that of wild-type cells (Figure S2C).

Altered centromeric chromatin promotes escape from mitotic arrest

Msc1 is known to have subtle effects on the behavior of centromeric heterochromatin (Lawrence and Volpe 2009; Qiu *et al.* 2010). Cells lacking *msc1* show a twofold increase in recruitment of the HP1-like H3 K9-methyl-binding protein Swi6 at pericentromeric heterochromatin (Lawrence and Volpe 2009), consistent with hypersilencing of marker genes placed within that region observed in *msc1Δ* cells (Qiu *et al.* 2010). Swi6 plays a direct role in chromosome segregation by recruiting the cohesin complex to pericentromeric chromatin (Nonaka *et al.* 2002) and has been suggested to function as a docking platform for Clr6 complex II and the SHREC complex (Nicolas *et al.* 2007; Sugiyama *et al.* 2007). Given that cohesion is impaired in *swi6Δ* cells, we asked whether *swi6Δ* could suppress the *dis1-288 cs* phenotype. As shown in Figure 3A, in the double mutant *swi6Δ dis1-288*, deletion of Swi6 had a minor impact on the *dis1-288* phenotype at 22°, suggesting that loss of function of Swi6 alone cannot suppress its *cs* phenotype. Given the observation that deletion of *msc1* results in increased amounts of Swi6 at centromeres (Lawrence and Volpe 2009), we predicted that *msc1Δ*-mediated suppression of *dis1-288* might depend on the reported increase in Swi6 recruitment at centromeres in the *msc1Δ* strain. Contrary to expectations, the triple mutant *msc1Δ swi6Δ dis1-288* grows more robustly at 22° than the double mutant *msc1Δ dis1-288*. Furthermore, deleting both *msc1* and *swi6* virtually eliminates the *dis* phenotype. Thus, wild-type Swi6 limits suppression of the *dis1* phenotype conferred by deletion of *msc1*.

As shown in Figure 3D, the *swi6Δ* and *msc1Δ swi6Δ* cells are normal in length. However, the double mutant *swi6Δ dis1-288* exhibits an elongated phenotype, suggesting a delay in cell-cycle progression that might reflect the reduced fitness at 22°. Remarkably, deletion of *msc1Δ* alleviates this phenotype because the length of the triple mutant *msc1Δ swi6Δ dis1-288* is in the normal range.

Targeted inhibition of HDAC function is necessary to promote survival of cells compromised for *dis1*

TSA is widely used as an HDAC inhibitor. Since elimination of particular HDAC proteins can suppress the *cs* phenotype of *dis1-288* cells, we tested whether chemical inhibition of HDAC activity might produce the same effect. As shown in

oval-end arrow (3:0), and diamond-end arrow (evenly distributed but hypercondensed). Scale bar, 5 μm. (C) Quantitation of the percentage of cells exhibiting the *dis* phenotype following incubation at 22° for 4 hr ($n > 150$). (D) Measurement of mean cell length of septated cells ($n > 30$), with SD shown in brackets. *Cells exhibit long phenotype (>15.6 μm); ^cells grown at 25°; ^cells grown at 28°.

Figure 2 Mad2 function is essential for *msc1Δ*-mediated suppression of the *dis1* phenotype. (A) Cell growth on day 5 at the indicated temperatures. (B) Representative images of cells grown to mid-log phase at permissive temperature (33°C) or shifted for 4 hr to 22°C. Fixed cells were stained with DAPI and photographed under a fluorescence microscope. Asterisks indicate cells exhibiting normal mitosis. Arrows indicate the *dis* phenotype (chromosomes condensed): open-end arrow (2:1), oval-end arrow (3:0), as well as a variation on the *dis* phenotype with unequally segregated decondensed chromosomes: closed arrow (2:1). Scale bar, 5 μm. Representative images corresponding to wild type, *dis1-288*, and *msc1Δ dis1-288* strains are shown in Figure 1B. (C) Quantitation of the percentage of cells exhibiting normal segregation, *dis* (condensed), *dis* (decondensed), or cut phenotype following incubation at 22° for 4 hr ($n > 150$).

Figure 4A, when *dis1-288* cells were plated in the presence of TSA at 22°, there was no suppression of the *cs* phenotype. Indeed, the *dis1-288* strain was hypersensitive to TSA because it is unable to grow in the presence of a low concentration of TSA (5 μg/ml) at 25°, a temperature that is typically permissive for *dis1-288* growth. Furthermore, the ability of *msc1Δ* to suppress *dis1-288* at 22° was lost in the presence of TSA, and growth of the *msc1Δ dis1-288* strain was compromised at higher temperatures in the presence of TSA. Thus, while targeted inhibition of particular HDAC activities in *dis1*-compromised cells can lead to cell survival (Figure 1), broad inhibition of HDAC function by TSA leads to nonviability of the *dis1-288* mutant (Figure 4A).

The type I HDAC Clr6 exists in at least three distinct complexes: complex I (Clr6, Prw1, Pst1, and Sds3), complex I' (all complex I components plus Png2), and complex II (Clr6, Prw1, Pst2, Cph1, Cph2, and Alp13) (Nicolas *et al.* 2007). A variation of complex I also has been described (Zilio *et al.* 2014). To test whether disruption of specific Clr6 complexes results in a phenotype similar to that seen with inactivation of *clr6-1* (Figure 1A), we constructed *dis1-288* double mutants with representative mutants of complex I' (*png2Δ*) and complex II (*pst2Δ*). As shown in Figure 4B, when compared to *clr6-1 dis1-288*, the double mutant strains *pst2Δ dis1-288* and *png2Δ dis1-288* were not viable at 22°. Thus, while impaired *clr6* function can suppress the *cs* phenotype of *dis1-288* mutant cells, disruption of particular Clr6 subunits is unable to exert a similar effect.

Loss of function of *Msc1* can promote cell survival when microtubules are compromised by exposure to thiabendazole

To determine whether the absence of *msc1* can affect cell survival when microtubule function is compromised by means other than *dis1* inactivation, we grew *msc1Δ* cells in

the presence of the microtubule-destabilizing drug TBZ. As shown in Figure 5A, deletion of *msc1* or *swr1* conferred resistance to TBZ at 25° compared to a wild-type strain. In contrast, of the four HDAC mutant strains, *clr6-1* and *sir2Δ* strains were more sensitive than wild-type cells, while *hos2Δ* and *clr3Δ* exhibited similar sensitivity. As reported previously by others (Ekwall *et al.* 1996), an *swi6Δ* strain is quite sensitive to TBZ (Figure 5A). As shown in Figure 5B, whereas *msc1Δ* increased resistance to TBZ in an *swi6+* background at 25°, simultaneous deletion of *msc1* and *swi6* resulted in sensitivity to TBZ equivalent to that of the *swi6Δ* mutant alone. Both *swi6Δ* and *msc1Δ swi6Δ* exhibited TBZ sensitivity that varied with temperature (compare Figure 5, B and C). Whereas at 25° *swi6Δ* cells were nonviable and the double mutant *msc1Δ swi6Δ* was hypersensitive to TBZ, at 32°, *swi6Δ* cells were partially sensitive to 10 μg/ml TBZ and *msc1Δ* could suppress this sensitivity of *swi6Δ* cells.

Discussion

Proteins that regulate epigenetic marks on histones are categorized as “writers,” such as histone acetyltransferases (HATs) and histone methyltransferases (HMTs); “erasers,” such as histone deacetylases (HDACs) and histone lysine demethylases (KDMs); and “readers,” such as proteins harboring chromodomains, bromodomains, and PHD domains that recognize lysine residues modified with particular molecular entities (Jenuwein and Allis 2001). Like its mammalian homologs, *Msc1* is comprised of both eraser (JmjC) and reader (PHD) domains. Unlike the mammalian KDM5A and KDM5B proteins, however, the JmjC domain of *Msc1* does not have all the residues thought to contribute to demethylase activity (Klose *et al.* 2006). Additionally, while the three PHD domains of *Msc1* suggest that it may behave as a reader of H3 lysine methyl marks, we were unable to detect binding of the

Figure 3 The presence of *swi6* limits *msc1Δ*-dependent suppression of *dis1* phenotype. (A) Growth of indicated strains at specified temperatures as documented on day 4. (B) Representative images of fixed cells stained with DAPI. Asterisks indicate cells exhibiting normal mitosis. Open-end arrows indicate *dis* phenotype (2:1). Scale bar, 5 μ m. (C) Quantitation of the percentage of cells exhibiting the *dis* phenotype following incubation at 22° for 4 hr ($n > 150$). (D) Measurement of mean cell length of septated cells ($n > 30$), with SD shown in brackets. [†]Cells exhibit long phenotype ($>15.6 \mu$ m). The measurements for wild-type, *msc1Δ*, and *dis1-288* are shown in Figure 1D.

Msc1 PHD domains to histone proteins (Dul and Walworth 2007) or methyl-modified histone peptides (C. T. Bernard and N. C. Walworth, unpublished data). We could, however, demonstrate association of Msc1 with the ubiquitin-conjugating enzyme Rhp6 and found that the PHD domains of Msc1 possess E3 ubiquitin protein ligase activity, a property shared with PHD domains of several other proteins (Dul and Walworth 2007).

As is true for human KDM5A (Nishibuchi *et al.* 2014), Msc1 associates with HDAC activity (Ahmed *et al.* 2004), although there is no evidence to date indicating that Msc1 exists in a stable physical complex with purified fission yeast HDACs Clr6, Clr3, and Sir2 (Nicolas *et al.* 2007; Sugiyama *et al.* 2007; Buchanan *et al.* 2009; Wang *et al.* 2013; Zilio *et al.* 2014). Further suggesting a role for Msc1 in chromatin biology, Msc1 has been found consistently as a component of the fission yeast Swr1C complex (Buchanan *et al.* 2009; Kim *et al.* 2009; Zofall *et al.* 2009; Hou *et al.* 2010; Qiu *et al.* 2010), which facilitates the exchange of histone H2A.Z for H2A at specific chromosomal locations. Cells lacking Msc1 exhibit elevated levels of histone H3 dimethyl-K9 at pericentromeric heterochromatin, consistent with persistence of the fission yeast HP1 homolog Swi6 in this region (Lawrence and Volpe 2009). This observation led to speculation that the histone H3 K9-methyl binding protein Swi6 could be a target

of Msc1 ubiquitin ligase activity (Braun and Madhani 2012), which, to our knowledge, has not yet been tested. Genetic evidence also suggests that Msc1 influences chromosome biology: elevated Msc1 copy number suppresses the *ts* phenotype of the *cnp1-1* mutant, which encodes a mutant allele of the centromeric histone H3 variant CENP-A, whereas deletion of *msc1* compromises the viability of a number of mutants encoding proteins involved in kinetochore function (Ahmed *et al.* 2007).

Mutations that alter centromeric chromatin promote cell survival in a mitotic-arrest mutant

Cold-sensitive mutants of Dis1, the fission yeast homolog of the microtubule-polymerizing protein XMAP215 (Al-Bassam and Chang 2011), display a phenotype in which sister chromatids fail to segregate properly at temperatures of 22° and below: chromosomes condense, the spindle forms and elongates, but sister chromatids fail to separate, a phenotypes known collectively as the *dis* phenotype (Ohkura *et al.* 1988). The molecular activity of the XMAP215 family of proteins and the phenotype of the *dis1* mutant suggest the reasonable hypothesis that the failure of sister-chromatid separation in the *dis1* mutant is due to a defect in association of spindle microtubules with the kinetochore such that the

Figure 4 Nonspecific disruption of HDAC activity or disruption of Clr6 complex subunits cannot suppress *cs* phenotype of *dis1-288* mutant cells. (A) Loss of function of *msc1* sensitizes cells with compromised microtubules to TSA. Cells growth on YES plates in the absence (–TSA) or presence of 5 μ g/ml TSA (+TSA) following incubation at specified temperatures for 5 days. (B) Disruption of noncatalytic Clr6 complex subunits cannot suppress *dis1*. Cell growth on day 5 at 22° (restrictive temperature for *dis1-288*), 33° (permissive temperature for *dis1-288*), or 36° (restrictive temperature for *clr6-1*). Corresponding single mutants shown in Figure S3.

SAC cannot be silenced. We reported previously that deletion of *msc1* in the *dis1* mutant allows *dis1* cells to survive at low temperature (Qiu *et al.* 2010). Dis1 shares an essential function in cell viability with Alp14, another member of the XMAP215/TOG family: while *dis1Δ* results in cold sensitivity, *alp14Δ* results in temperature sensitivity, and *alp14Δ dis1Δ* double deletions are nonviable (Garcia *et al.* 2001). While *msc1Δ* can suppress the *cs* phenotype of *dis1Δ* cells (Figure S1A), *msc1Δ* compromises the *ts* phenotype of *alp14Δ* cells (Figure S1B), consistent with a genome-wide genetic interaction map reporting a negative genetic interaction between *msc1Δ* and *alp14Δ* (Roguev *et al.* 2008). Thus, the *msc1Δ*-mediated suppression owing to improvement in improper kinetochore-microtubule attachment caused by mutants of *dis1-288/dis1Δ* likely stems from Dis1-specific functions as opposed to functions shared with Alp14.

Given that Msc1 associates with HDAC activity (Ahmed *et al.* 2004), we have now tested whether mutations in fission yeast HDAC-encoding genes also can confer survival on *dis1* cells. We observed that combining individual mutations of *clr6-1*, *clr3Δ*, or *sir2Δ* with *dis1-288* conferred cell survival on the double-mutant strains at temperatures restrictive for the single *dis1-288* mutant. Strikingly, Swi6, which maintains pericentromeric regions as heterochromatin through binding to methylated K9 on histone H3 (Partridge *et al.* 2000; Grewal and Jia 2007), limited suppression of *dis1-288* caused by loss of *msc1* function: combined loss of *msc1* and *swi6* virtually eliminated the *dis* phenotype in the *dis1-288* strain (Figure 3). As summarized in Figure 6, our results suggest that under conditions for which a compromised interaction between microtubules and the kinetochore leads to SAC activation, mitotic arrest, and cell death, the presence of additional mutations that alter the epigenetic state of centromeric chromatin can correct the *dis* phenotype and promote cell survival. Given that Clr6, Clr3, and Sir2 play key roles in heterochromatin assembly (Pidoux and Allshire 2005; Alper

et al. 2013; Buscaino *et al.* 2013), we interpret these results to suggest that alterations in centromeric chromatin may create a permissive chromatin landscape that allows interactions of microtubules with the kinetochore such that the SAC is silenced and sister chromatids separate. Because Swi6 is thought to recruit cohesin to centromeres (Partridge *et al.* 2002), the absence of *swi6* may lead to loss of cohesion and further alterations in centromere architecture that favor silencing of the SAC, thereby promoting sister-chromatid separation.

The histone deacetylase activity of Clr6 is histone H3 K9 specific (Bjerling *et al.* 2002). Clr3 histone deacetylase activity is linked to modification of multiple residues, including H3-K9, H3-K14, H3-K56, H4-K5, H4-K8, H4-K12, and H4-K16 (Kato *et al.* 2013). Sir2 HDAC activity is associated with deacetylation of H3-K4, H3-K9, H3-K14, and H4-K16 (Shankaranarayana *et al.* 2003; Wiren *et al.* 2005; Alper *et al.* 2013). We have reported previously that Msc1 contributes to maintenance of the deacetylated state of H3-K9 and/or H3-K14 (Ahmed *et al.* 2004). Thus, deletion of *msc1* or mutation of any of these three HDACs leads to varying degrees of elevated H3-K9 or H3-K14 acetylation and relieves mitotic arrest of the *dis1* mutant (Figure 1). We propose that alteration of the chromatin landscape at the centromere resulting from changes to acetylation of H3-K9 and/or H3-K14 allows for kinetochore-microtubule attachments in the *dis1* mutant that lead to SAC silencing and escape from mitotic arrest.

SAC function is essential for *dis1-288* mutant survival

The lagging chromosome phenotype, which is common to *msc1Δ* cells and mutants that affect heterochromatin (Ahmed *et al.* 2004; Pidoux and Allshire 2004), has been attributed to an elevated frequency of merotelic interactions between chromosomes and the mitotic spindle (Courthouex *et al.* 2009; Rumpf *et al.* 2010). Merotelic interactions are those in which one kinetochore of a chromosome pair attaches to a microtubule derived from one spindle pole, while the other

Figure 5 TBZ resistance of *msc1Δ* cells requires intact heterochromatin at 25°. Cell growth on YES plates on the indicated strains in the absence (0 μg/ml) or presence (10 μg/ml) of TBZ following incubation at (A) 25° for 3 days, (B) 25° for 5 days, and (C) 32° for 3 days.

kinetochore of the pair attaches to microtubules derived from both spindle poles. Because both kinetochores are attached to microtubules, such interactions do not activate the SAC (Cimini *et al.* 2001). Perhaps merotelic associations, revealed as the lagging chromosome phenotype and promoted by loss of HDAC or *msc1* function, favor SAC silencing, which in the context of limited (or absent) *dis1* function allows sisters to dissociate. Supporting this explanation, we report that the SAC must be intact for deletion of *msc1* to support suppression of *dis1-288*: the observed suppression of the *dis1-288* mutant in combination with *msc1Δ* is completely lost in the triple mutant *msc1Δ mad2Δ dis1-288* (Figure 2). Consistent with previous observations that Mad2 function is essential for viability of cells with a compromised mitotic spindle, Mad2 is necessary for cell survival when *dis1* is compromised (Figure 2) because survival of *dis1-288* cells is very limited, and a double mutant could only be isolated under favorable conditions (Figure S2). These observations lead us to speculate that the observed suppression of *dis1-288* by mutants

that alter the landscape of centromeric chromatin relies on a mechanism that leads to SAC activation and subsequent silencing to allow sister chromatids to separate successfully (Figure 6).

Msc1 may function in localizing HDAC activity to centromeres

The C-terminal PHD fingers of Msc1 associate with HDAC activity *in vitro* (Ahmed *et al.* 2004). Our epistasis analysis suggests that loss of function of Msc1 and loss of function of HDAC activity, specifically Clr6, Clr3, or Sir2, can suppress the *dis1-288* mutant strain. Several possible mechanisms have been suggested to account for Clr6 recruitment to the centromere, including association with CENP-B, a sequence-specific DNA binding protein, or recruitment via sequence-independent epigenetic modifications and the heterochromatin-associated HP1 protein Swi6 (Nakagawa *et al.* 2002; Grewal and Jia 2007). A PHD domain-containing protein ING2 has been implicated in targeting the mSin3A-HDAC complex by binding di- or trimethylated H3-K4 (Shi *et al.* 2006). Based on the presence of three PHD domains in Msc1 and the ability of the C-terminal PHD domains to recruit HDAC activity *in vitro*, it is possible that Msc1 may act to localize HDAC activity. The similarity in phenotype of suppression of *dis1-288* by *msc1Δ* or loss of HDAC function is consistent with this model. Nonetheless, attempts to co-immunoprecipitate Msc1 with Clr6 have been unsuccessful (S. Ahmed and N. C. Walworth, unpublished data). Notably, widespread elimination of HDAC function via treatment of cells with the HDAC inhibitor TSA does not restore viability to *dis1-288* (Figure 4A).

Work in numerous organisms has given rise to working models for organization of the eukaryotic kinetochore. The

Microtubule Dysfunction- *dis1-288* mutant

Figure 6 Model summarizing genetic observations. Alterations in the centromeric chromatin environment can compensate for microtubule dysfunction by allowing for successful kinetochore-microtubule interactions that satisfy the SAC. At low temperatures, *dis1-288* cells exhibit lethal mitotic arrest (left panel). Combining *msc1Δ* with *dis1-288* can suppress *dis1-288* mitotic arrest by promoting successful kinetochore-microtubule interactions (center panel), thereby satisfying the SAC. Additional loss of function of *swi6* further alters centromeric chromatin architecture, allowing for complete suppression of the mitotic defect, which results in normally growing cells (right panel).

CCAN, a complex of more than a dozen conserved proteins, forms the inner kinetochore, which associates with centromeric chromatin (McAinsh and Meraldi 2011; Westhorpe and Straight 2015). An outer kinetochore network, named KMN for its three major constituents, Knl1, the Mis12 (or MIND) complex, and the Ndc80 complex, mediates the association of the CCAN with spindle microtubules (Cheeseman *et al.* 2006; Santaguida and Musacchio 2009; Foley and Kapoor 2013). Structural studies have begun to elucidate the relationship between components of these complexes (Alushin *et al.* 2010; Petrovic *et al.* 2014). The heterotetrameric Ndc80 complex interacts with microtubules through calponin homology domains in Ndc80 and Nuf2 and with the Mis12 complex through SPC24 and SPC25 (Wei *et al.* 2005; Wei *et al.* 2007). Fission yeast Dis1 binds to a loop domain on the NDC80 protein (Hsu and Toda 2011), placing it in an ideal location to exert its enzymatic activity and influence microtubule polymerization (Brouhard *et al.* 2008). While genetic studies of the sort reported here are unable to reveal mechanistic relationships between protein complexes, they may inform refinement of those models and suggest biochemical tests of them. Because the *dis1* mutant arrests in mitosis, we interpret suppression of mitotic arrest as an indication that mutations in the HDACs or loss of function of *msc1* allows kinetochore-microtubule interactions to occur that silence the mitotic checkpoint and allow chromosomes to separate. The requirement for Mad2 for suppression is consistent with this model. Thus, it is intriguing to note that similar to loss of function of the HDACs or *msc1*, a *ts* mutant of the essential *mis12* gene is reported to suppress the *cs* phenotype of a *dis1* deletion (identical in phenotype to *dis1-288*), although the double mutant exhibits synthetic lethality at higher temperatures typically permissive for either single mutant (Goshima *et al.* 1999). Thus, it is possible that the complex interactions that occur at the interface of chromosomes and the microtubules of the mitotic spindle have built in some degree of flexibility to maximize the opportunity for securing chromosome segregation under nonideal conditions. This flexibility is not without risk, however, because HDAC mutants, like cells lacking *msc1*, exhibit chromosome segregation defects.

Codependent function of Msc1 in chromosome segregation

A role for Msc1 in chromosome segregation has become apparent as a result of both gain- and loss-of-function genetic interactions with various centromere and kinetochore mutants (Ahmed *et al.* 2004, 2007; Dul and Walworth 2007; Qiu *et al.* 2010). A recent study suggests a role for a Msc1-mediated pathway in establishing neocentromeres in fission yeast (Ogiyama *et al.* 2013). Better understanding the role of Msc1 in chromosome segregation could have implications for elucidating the functions of the mammalian cancer-associated proteins KDM5A and KDM5B that share similar domain architecture with Msc1. KDM5A (JARID1A) was originally discovered as the retinoblastoma binding protein RBP2 (Fattaey *et al.* 1993) that colocalizes with the retinoblastoma (Rb) complex (Benevolenskaya

et al. 2005). KDM5A also interacts physically and functionally with the Polycomb complex, the Sin3 complex, and the Notch/RBP-J complex corepressor (Pasini *et al.* 2008; van Oevelen *et al.* 2008; Liefke *et al.* 2010). Overexpression of KDM5A is linked to myeloid leukemia and gastric and cervical cancer (Blair *et al.* 2011). Overexpression of KDM5B (JARID1B), originally described as PLU-1, a gene upregulated in breast cancer (Lu *et al.* 1999), has been associated with cancers of the ovary, testis, prostate, bladder, and esophagus, as well as melanoma (Barrett *et al.* 2002; Xiang *et al.* 2007; Yamane *et al.* 2007). In human cancer cell lines, a KDM5A-dependent altered chromatin state results in drug-tolerant cells (Sharma *et al.* 2010). Our studies of Msc1, the fission yeast homolog of the KDM5 family of proteins, are consistent with a role for Msc1 in mediating chromosome segregation, perhaps by modifying the chromatin landscape at the centromere. Mitotic defects in cells lacking the tumor suppressor Rb, including merotelic attachments and lagging chromosomes (Manning *et al.* 2010), suggest that Rb itself contributes to proper chromosome segregation (Manning and Dyson 2012), perhaps via effects on chromosome cohesion (Manning *et al.* 2014). These results, along with the observations reported here, suggest that an examination of the role of KDM5 proteins in chromosome segregation is warranted as well. Because the KDM5 proteins have multiple functional domains potentially targetable by small molecules, it would be prudent to consider the possible consequences of targeting KDM5 proteins on genomic stability in mammalian systems.

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Escape from Mitotic Arrest: An Unexpected Connection Between Microtubule Dynamics and Epigenetic Regulation of Centromeric Chromatin in *Schizosaccharomyces pombe*

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Table S1 Fission yeast strains used in this study

Strain	Genotype	Source
SP6	<i>h⁻ leu1-32</i>	Lab collection
NW2908	<i>h⁻ dis1-288 leu1-32</i>	This study
NW2739	<i>h⁻ msc1::hyg^R leu1-32</i>	Lab collection
NW1622	<i>h⁺ clr6-1 ura4-D18 ade6-216</i>	Lab collection
NW2909	<i>h⁻ clr3::kan^R leu1-32</i>	This study
NW2910	<i>h⁺ hos2::kan^R leu1-32 ura4-D18 ade6-216</i>	Lab collection
NW2911	<i>h⁻ sir2::kan^R leu1-32 ura4-D18 ade6-210</i>	This study
NW2638	<i>h⁺ msc1::kan^R dis1-288 leu1-32</i>	Lab collection
NW2912	<i>h⁺ clr6-1 dis1-288 ura4-D18 ade6-216</i>	This study
NW2913	<i>h⁻ clr3::kan^R dis1-288 leu1-32</i>	This study
NW2914	<i>h⁺ hos2::kan^R dis1-288 leu1-32 ura4-D18 ade6-216</i>	This study
NW2915	<i>h[?] sir2::kan^R dis1-288</i>	This study
NW2917	<i>h⁺ pst2::kan^R leu1-32 ura4-D18 ade6-216</i>	Lab collection
NW2918	<i>h⁺ png2::kan^R leu1-32 ura4-D18 ade6-216</i>	Lab collection
NW2919	<i>h[?] cph1::kan^R dis1-288 leu1-32 ura4-D18</i>	This study
NW2920	<i>h[?] pst2::kan^R dis1-288 leu1-32 ade6-216</i>	This study
NW2921	<i>h[?] png2::kan^R dis1-288 leu1-32</i>	This study
NW1564	<i>h⁻ msc1::kan^R leu1-32</i>	Lab collection
NW1419	<i>h⁻ mad2::ura4⁺ leu1-32 ura4-D18 ade6-210</i>	Lab collection
NW2922	<i>h⁻ mad2::ura4⁺ dis1-288 leu1-32 ura4-D18</i>	This study
NW2923	<i>h⁺ msc1::kan^R mad::ura4⁺ leu1-32 ura4-D18 ade6-210 his7-366</i>	This study
NW2924	<i>h⁺ msc1::kan^R dis1-288 mad::ura4⁺ leu1-32 ura4-D18 his7-366</i>	This study
NW2925	<i>h⁻ swi6::kan^R leu1-32 ade6-216</i>	This study
NW2926	<i>h⁺ swi6::kan^R msc1::hyg^R leu1-32</i>	This study
NW2927	<i>h⁻ swi6::kan^R dis1-288 leu1-32 ura4-D18</i>	This study
NW2928	<i>h⁹⁰ swi6::kan^R msc1::hyg^R dis1-288 leu1-32</i>	This study
NW2937	<i>h⁻ alp14::ura4⁺ ura4-D18 leu1-32</i>	YGRC, Japan FY17702
NW2938	<i>h⁺ dis1::ura4⁺ ura4-D18 leu1-32 lys1-131</i>	YGRC, Japan FY8617
NW2939	<i>h⁺ msc1::hyg^R ura4-D18 leu1-32</i>	Lab Collection
NW2940	<i>h[?] msc1::hyg^R dis1::ura4⁺ ura4-D18 leu1-32 lys1-131[?]</i>	This study
NW2941	<i>h[?] msc1::hyg^R alp14::ura4⁺ ura4-D18 leu1-32</i>	This study

Figure S1

(A) *msc1Δ* suppresses *dis1Δ* cold sensitive phenotype.

The indicated strains were incubated at specified temperatures were documented on day 7 for 22°C, day 5 for 25°C and day 5 for 33°C .

(B) *msc1Δ* compromises growth of *alp14Δ* mutant cells. The indicated strains were incubated at specified temperatures and documented on day 4.

(C) Single mutants corresponding to figure 1A were grown at specified temperatures and documented on day 5.

A. Random Spore Analysis

Genotype	30°C	33°C	36°C*
<i>mad2Δ dis1-288</i>	0%	0%	10%*
<i>mad2Δ dis1⁺</i>	21%	31%	27%
<i>dis1-288 mad2⁺</i>	44%	31%	29%
<i>dis1⁺ mad2⁺</i>	35%	38%	35%

* at 36°C by random spore analysis observed a few sick double mutants
n=104 segregants tested at each temperature

B. Tetrad Analysis

Representative result at 30°C, 33°C and 36°C

C.

Strain	33°C	22°C
<i>mad2Δ dis1-288</i>	12.6 (1.0)	12.3 (1.4)
<i>msc1Δ mad2Δ dis1-288</i>	13.3 (0.9)	13.2 (0.8)
<i>msc1Δ mad2Δ</i>	13.4 (0.6)	12.9 (0.9)
<i>madΔ</i>	13.4 (0.8)	13.2 (0.8)

Figure S2 *mad2Δ* and *dis1-288* exhibit synthetic lethal or synthetic growth defect genetic interactions depending on the temperature at which the cross is performed and screening method. *mad2Δ* strains were crossed with *dis1-288* at 30°C, 33°C (permissive for *dis1-288*) and 36°C, and were screened by both Random Spore Analysis (A) and Tetrad Analysis (B) C. Measurement of mean cell length of septated cells (n>30), with standard deviation (S.D.) shown in brackets.

Figure S3 Single mutants corresponding to figure 4B were grown at specified temperatures and documented on day 5.