**Movies 1 and 2.** Time-lapse images of \textit{mug28}Δ GFP-Psy1 (movie 1) and \textit{mug28}+ GFP-Psy1 (movie 2) cells in live meiotic cells. Cells were observed from metaphase II to anaphase II. Images are recorded at 3 minute intervals.

**Movies 3 and 4.** Time-lapse images of \textit{mug28}Δ Meu14-GFP (movie 3) and \textit{mug28}+ Meu14-GFP (movie 4) in live meiotic cells. Cells were observed from metaphase II to anaphase II. Images are recorded at 3 minute intervals.

**Movies 5 and 6.** Time-lapse images of \textit{mug28}Δ Meu14-GFP GFP-Psy1 (movie 5) and \textit{mug28}+ Meu14-GFP GFP-Psy1 (movie 6) in live meiotic cells. Cells were observed from metaphase II to anaphase II. Images are recorded at 3 minute intervals.
**Mug28, a meiosis-specific protein of *Schizosaccharomyces pombe*, regulates spore wall formation**

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**SUPPLEMENTARY DATA**

**SUPPLEMENTARY MATERIALS AND METHODS**

*Live Cell Observation*

To detect Mug28-GFP, Meu10-GFP, and mCherry-Psy1 in living meiotic cells, mid-log-phase *mug28*+GFP (AS181) and *meu10*+GFP *mCherry-psy1*+ (NP250 and 251) cells were cultured in EMM2-N medium to induce meiosis as described previously (Saito et al., 2005). The living cells were then stained with 3.0 μg/ml Hoechst33342 for 5 min and spotted on a coverslip. Fluorescence images of these cells were observed using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) with a Cool SNAP charge-coupled-device camera (Roper Scientific, San Diego, CA). Fluorescence images were acquired using Adobe Photoshop 7.0.

*Gene Disruption of mug28+

To disrupt the *mug28* gene by replacing it with the *ura4* gene, we used the polymerase chain reaction (PCR) to amplify a DNA fragment carrying the 5' upstream and 3' downstream regions of the *mug28* gene. For this purpose, we synthesized the following four oligonucleotides and used them as primers (see Table S2 for their sequences): mug28-5’Fw-KpnI, mug28-5’Rv-XhoI, mug28-3’Fw-XhoI-stop-PstI and mug28-3’Rv-SacI’. These PCR products and the 1.8 kb *HindIII* fragment containing the *ura4* gene were inserted into the pBluescriptII KS (+) vector via KpnI-XhoI, PstI-Sacl or *HindIII* sites. The mug28-5’Fw-KpnI and mug28-3’Rv-SacI primers generated a 2.8 kb PCR product containing the *ura4* cassette. This plasmid construct was digested with KpnI and SacI, and the resulting construct was introduced into the *h*+ wild-type strain AS6 (*h*+ ade6-M210 leu1-32 ura4-D18). The Ura+ transformants were then screened by PCR analysis to identify the disrupted strain.
Western Blot Analysis
For western blot analysis, $2.8 \times 10^8$ *S. pombe* cells were suspended in 0.4 ml of 20% trichloracetic acid (TCA) solution. The cells were disrupted with acid-washed glass beads using a vortex mixer, and then a 5% TCA solution was added and the total protein precipitates were obtained by centrifugation. The protein precipitate was then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto a polyvinylidene difluoride membrane (Immobilon, Millipore, Bedford, MA). These blots were probed with a rat anti-HA polyclonal antibody and the bands were visualized using the Renaissance chemiluminescence system (NEN Life Science, Boston, MA).

Thin-section Electron Microscopy (EM) Observations
Cells were incubated on ME plates at 28°C for 18 h, collected, mounted in a thin layer on a copper grid, and immersed in liquid propane cooled with liquid nitrogen (Leica EM CPC; Leica Microsystems, Vienna, Austria). The frozen cells were transferred to 2% OsO$_4$ in dry acetone and kept at −80°C for three days. Thereafter, they were gradually warmed from −80°C to 0°C over 5 h, held for 1 h at 0°C, and then warmed from 0°C to 23°C (room temperature) for 2 h (Leica EM AFS; Leica Microsystems). After being washed with dry acetone three times, the samples were infiltrated with increasing concentrations of Spurr's resin in dry acetone and finally with 100% Spurr's resin. After polymerization, ultrathin sections were cut by an ultramicrotome (Leica Ultracut UCT; Leica Microsystems) and stained with uranyl acetate and lead citrate. The sections were viewed on an electron microscope (H-7600; Hitachi Co., Tokyo, Japan) at 100 kV.

Spore wall thickness was measured at five, five, five and ten places (n=25) for four independent *mug28*+ spore walls (see Figures 7F and S7), and their average value was calculated (see Figure 7G). Similarly, spore wall thickness was measured at seven, seven, eight, and nine places (n=31) for four independent *mug28*Δ spore walls (see Figures 7F and S7), and their average value was calculated (see Figure 7G).

Construction of Mug28 Mutants
For efficient construction of various deletion mutants, we first prepared a DNA fragment named 5’UTR+ *mug28*+ORF(1-30)+SalI by PCR that contains the 5’UTR and open reading frame (ORF) of *mug28*+ (1-30) with the SalI site at its 3’ end, using the 5’UTR-Fw-KpnI and the inORF-plus-SalI-Rv primers (Table S2). Similarly, we prepared the SalI+ *mug28*+ORF(31-1149) fragment by PCR using inORF-plus-SalI-Fw and inORF-BamHI-Rv primers harboring the KpnI, SalI and BamHI sites (Table S2).
Then, plasmid DNA harboring the 5’UTR+mug28+ ORF(1-30)+SalI fragment was digested with KpnI/SalI, and the liberated fragment was ligated with the SalI+mug28+ ORF(31-1149) fragment to generate the pT7Blue5’UTR+mug28+ ORF(1-30)+SalI+mug28+ ORF(31-1149) fragment. This fragment was subsequently ligated to the mug28+ ORF (1149-) gfp-3’UTR fragment that was BamHI/SacI digested from plasmid pBluScriptII KS(+)+Ura4+ mug28+ ORF(-EcoRI 772)-gfp-3’UTR, which was used to prepare the AS108 (h90 ade6-M210 leu1-32 ura4-D18 mug28+::mug28+ gfp) strain. Then, the generated pT7Blue +5’UTR+mug28+ ORF(+SalI)-gfp-3’UTR was digested with KpnI/SacI to release the 5’UTR+mug28+ ORF(+SalI)-gfp-3’UTR fragment, which was inserted into pJK148 to obtain the basic plasmid, pJK148 5’UTR+mug28+ ORF(+SalI)-gfp-3’UTR, for deletion of mutants-GFP.

To prepare the RRM1Δ strain, we performed PCR with the 5’UTR-Fw-KpnI and the RRM1Δ-SpeI-Rv primers (Table S2), using the basic plasmid as a substrate. The generated DNA fragment was digested with KpnI/SpeI to obtain the RRM1Δ fragment, which replaced the RRM1 region of the basic Mug28 construct. For RRM2Δ, we performed PCR using the inORF-plus-SalI-Fw (described above) and the RRM2Δ-BamHI-Rv primers harboring the BamHI site (Table S2). The SalI/BamHI digest of the amplification product replaced the RRM2 region of the basic Mug28 construct. For RRM3Δ, the inORF-plus-SalI-Fw primer (described above) and RRM3Δ-NotI-Rv primer, harboring the NotI site, were used for PCR amplification, and the amplification product was digested with SalI/NotI to replace the RRM3 region of the basic Mug28 construct. For RRM1ΔRRM2Δ, the RRM3O-SalI-Fw primer, with the SalI site underlined, and the mug28ORF-3’Rv-NotI (nonstop codon) primer (described above) were used for PCR amplification, and the amplification product was digested with SalI/NotI for replacement of the RRM1/RRM3 region of the basic Mug28 construct. For RRM2ΔRRM3Δ, the inORF-plus-SalI-Fw primer (described above) and the RRM1O-NotI-Rv primer (Table S2). For RRM1ΔRRM3Δ, the RRM2O-SalI-Fw and RRM3Δ-NotI-Rv primers harboring the NotI, SalI, and NotI sites (Table S2), were used for PCR amplification, and the amplification products were digested with SalI/NotI to replace the RRM1/RRM3 region of the basic Mug28 construct. Subsequently, these constructs were digested with NruI and introduced into AS99 (h90 ade6-M210 leu1-32 ura4-D18 mug28+::ura4+). We screened the Leu+ transformants and confirmed the precise integration of constructs by PCR and digestion with relevant restriction enzymes.

To prepare the Mug28F466A-GFP strain, we synthesized a mug28+ ORF fragment
harboring the BamHI and NotI sites. Then, the plasmid named pJK148 5’UTR+mug28+ORF(+SalI)-gfp-3’UTR was digested with BamHI/NotI, and the liberated fragment was exchanged with the BamHI+mug28+ F466A fragment to generate the pJK148 5’UTR+mug28F466A ORF(+SalI)-gfp-3’UTR plasmid. Subsequently, these constructs were digested with NruI and introduced into AS99 (h^90 ade6-M210 leu1-32 ura4-D18 mug28^+::ura4^+). Then, we screened the Leu^+ transformants and confirmed the precise integration of the constructs by PCR and digestion mapping with relevant restriction enzymes.

We also prepared 3HA-tagged deletion mutants using a similar process. Briefly, the GFP-mug28^+3’UTR fragment was digested with NotI/SacI from the plasmid used for each mutant-GFP construct and exchanged with the 3HA-mug28^+3’UTR fragment of the pREP41-3HA-mug28^+ 3’UTR plasmid. Then, we obtained the HA-basic plasmid, pJK148 5’UTR+mug28^+ORF(+SalI)-3ha-3’UTR, and constructed the 3HA-tagged deletion mutants. Subsequently, these constructs were digested with NruI and introduced into AS99 (h^90 ade6-M210 leu1-32 ura4-D18 mug28^+::ura4^+). Then, we screened the Leu^+ transformants and confirmed the precise integration of constructs by measuring the size of the DNA fragments that were generated by PCR amplification and subsequent digestion with relevant restriction enzymes. We mated these mutant 3HA-tagged strains with AS193 (h^+ ade6<<YFP-psy1 his2 leu1-32 ura4-D18), screened for Ade^+ transformants and established the mutant strains.

**Spore Viability**

Spore viability was determined as described previously (Kasama et al., 2006). Briefly, haploid parental strains were grown on YPD plates at 33°C. Cells were then mated and sporulated on SPA plates at 28°C (which promoted zygotic meiosis). After three to four days of incubation, the spores were treated with 1% glusulase (PerkinElmer Life Science, Inc.) for 3 h at room temperature and examined under a microscope for complete digestion of contaminating vegetative cells. The glusulase-treated spores were then washed with water and used to measure spore viability. The spores were separated on YPD agar plates using a micromanipulator (Singer Instruments, Somerset, UK). The plates were incubated at 33°C for three days before spore viability was measured.

**Construction of the Meu14DD-GFP Strain and Site-directed Mutagenesis**

To construct the Meu14DD-GFP mutant strain, the conserved Arg and Leu within two putative destruction boxes (RxxL) of Meu14-GFP were substituted by alanine by site-directed mutagenesis using PCR overlap extension (Weisman and Choder, 2001).
This generated plasmids expressing 5’UTR+-meu14DD-gfp-3’UTR with alanine substitutions at R148, L151, R161 and L164. Briefly, the pNP-meu14+’-3’UTR plasmid served as a template in a PCR reaction using the primer pair FN and D1-encode (5’-CGCTCGTTGATGTGCTGAGGGGTGGAT-3’), and the primer pair D1-code (5’-CAGGAGCAATGCACAACGAGCGATCTCAAGCAT-3’) and FC that contained the R148AL151A mutations. The resulting PCR product was purified by agarose gel electrophoresis (AGE) and used as a template for a second round of PCR with the primer pair FN and FC. Next, the resulting ORF of the meu14 PCR product with two alanine substitutions was purified by AGE and used as a template with primers FN and D2-encode (5’-CGCAGGATCTGCTTCCTCCTCAGTTCAATGCT-3’) and primers D2-code (5’-GCTGAGGAGAACAGATCTGCTCCCTAAACTTAC-3’) and FC that contained the R161AL164A mutations. The pNP-meu14+-3’UTR plasmid was replaced by this PCR fragment via NdeI and NotI sites. The resulting meu14DD-gfp DNA fragment carrying about 1 kb of both the upstream and downstream regions of meu14DD was digested with PstI and KpnI, and then used to transform meu14DD+::ura4+ cells (YDO100). Ura- transformants were obtained from the cells selected on 5-Fruoroorotic Acid (5-FOA) medium and confirmed by Southern blot analysis (data not shown). After confirming the mutations by DNA sequence analysis, we crossed the Meu14DD strain with the GFP-psy1+ strain (YN68) to make GFP observations (Figure S6).

**DNA Microarray Analysis**

Meiotic cells were collected at each time point (0, 4 and 8h) after nitrogen starvation. For monitoring the meiotic expression profiles of pat1 and pat1mug28Δ cells, DNA microarrays constructed by ASPEX corporation (Kanagawa, Japan) were used as described previously (Sugino et al., 2009). For each experiment, 50 μg of total RNA was converted into either Cy3- (wild type and mug28Δ cells at 0 h) or Cy5- (wild type and mug28Δ cells at 4 h and 8 h) labeled cDNA using a reverse transcriptase reaction kit (TaKaRa Bio Co. Ltd., Ohtsu, Japan) with oligo-dT as the primer. Microarray analysis were performed based on two-color experiments, namely, RNA from cells at 0 h time point was labeled with Cy3, while RNA from meiotic cells at each time point was labeled with Cy5. Hybridized microarrays were scanned for Cy3- and Cy5-labeled targets using a GenePix 4000B scanner (Axon Instruments, Union City, CA) with a resolution of 10 μm. Signal quantification for each probe on the microarray was performed with GenePix acquisition software (version 4.0, Axon Instruments). Clustering and visualization was conducted using GeneSpring GX 10.0.2 (Agilent
SUPPLEMENTARY RESULTS

DNA Microarray Analysis of Meiotic mug28Δ Cells
To examine if the RNA binding protein Mug28 has a role in transcriptional regulation, we performed transcriptome analysis of mRNAs from WT and mug28Δ cells during meiosis using a homemade DNA microarray of S. pombe (Sugino et al., 2009). For this purpose, we induced pat1 (JZ670) and pat1 mug28Δ (AS116) cells to enter meiosis synchronously and prepared RNA from cells at 0, 4 and 8 h after meiotic induction (blue arrows in Figure S2A). We compared the mRNA levels at 4 h vs 0 h and at 8 h vs 0 h in each RNA sample by using two-color microarray analysis, which allowed us to obtain a clustered heat map for 3,616 genes out of the 5,130 genes analyzed (Figure S2B-i). The results showed no notable changes in the mRNA levels for most S. pombe genes in mug28Δ cells during meiosis. Among the genes that were meiotically up-regulated (Figure S2B-ii) or down-regulated (Figure S2B-iii) in mug28Δ cells compared to mug28+ cells, an uncharacterized gene termed meu6+ (green arrow) showed the most conspicuous difference in expression. Other known meiotically induced genes (Figure S2B-iv) or those tagged with the gene ontology term “expressed during meiosis” in the S. pombe Gene DB (Figure S2B-v) did not show a more conspicuous difference in expression than meu6+ (green arrow). Previously reported spore wall related genes (Figure S2B-vi) or those tagged with the gene ontology term “ascospore-type prospore formation” in the S. pombe Gene DB (Figure S2B-vii) also failed to show a more conspicuous difference in expression. The entire processed data sets and each fold change value (Figures S2B and S2C) are presented in Table S3. As for the meu14+ gene (red arrow), the mRNA level of this gene was slightly higher than in the WT at 4 h, but this difference had almost disappeared at 8 h (Figure S2C). Thus, the residual Meu14-GFP signal (Figures 4 and 5) and Meu14-GFP proteins (Figure 4E) in mug28Δ cells were not due to the reduced mRNA level of meu14+. Since expression of meu6+ is upregulated at the late stage of meiosis (Watanabe et al., 2001), and its expression is largely induced only in mug28Δ cells, its function will be analyzed in a future study.

Over-expression of Meu14 Failed to Mimic the Phenotype of mug28Δ Cells
To examine if the residual amount of Meu14 protein at the tip or neck of the snowman-like FSM observed in mug28Δ cells is an underlying cause of the abnormal
phenotype of these cells, we constructed Psy1-GFP strains that over-expressed Meu14-9myc protein under the nmt1, nmt41 or nmt81 promoter, which enables strong, intermediate or weak expression after 18 h of depletion of thiamine in the medium. We incubated these strains on EMM plates in the presence of thiamine. Then, the colonies were transferred onto an EMM plate without thiamine and a nitrogen source to induce Meu14-9myc expression and meiosis. After 18 h incubation, we observed the Psy1-GFP signals in cells at the sporulation stage, when Meu14-9myc had reached maximum expression. We found that over-expression of Meu14 under these nmt promoters generated the snowman-like phenotype in only a very small number of cells (Figure S3B). This indicates that the over-expression of Meu14 alone failed to mimic the phenotype of mug28Δ cells.

**Phenotype of mug28Δmeu14Δ cells**

To examine for a genetic interaction between mug28+ and meu14+ genes, we compared the phenotypes of mug28Δ, meu14Δ and mug28Δmeu14Δ cells by observing the morphology of the FSM with Psy1-GFP signals. We found that most of the mother ascospores with a budded or snowman-like morphology in meu14Δ and mug28Δmeu14Δ cells were deformed (red or yellow arrowheads, respectively), whereas those of mug28Δ cells looked round and healthy like WT cells (Figure S4A). Moreover, the frequency of budded or snowman-like morphologies in meu14Δ and mug28Δmeu14Δ cells was lower than in mug28Δ cells (Fig. S4B). Furthermore, the fragmented nuclei detected in meu14Δ cells (Okuzaki et al., 2003) were not observed in mug28Δ cells, and the frequency of fragmented nuclei was not altered in mug28Δmeu14Δ cells (red bars in Figure S4B). These results show that the phenotypes of meu14Δ and mug28Δ cells are different and that these phenotypes do not affect each other, indicating the absence of a genetic interaction between mug28+ and meu14+ genes in these backgrounds.

**Ectopic Expression of Meu14DD Mutant**

To examine if the abnormal FSM formation observed in mug28Δ cells was due to dysregulation of timely degradation of Meu14 at the end of meiosis, we constructed mutant strains that expressed Meu14DD-GFP proteins (Meu14DD-R148AL151A or -R161AL164A), in which conserved Arg and Leu within the two putative destruction boxes (RxxL) were substituted by Ala, and Psy1-GFP for visualization of FSM. An examination of the behavior of FSMs during meiosis under a fluorescence microscope showed no conspicuous difference between the images of Meu14-GFP Psy1-GFP
(NP234) and Meu14DD-GFP Psy1-GFP (NP230) signals from Meiosis II to the sporulation stage (Figure S6A, left and right panels). Nonetheless, it is notable that Meu14DD-GFP/Psy1-GFP signals at the FSM looked stronger than those of Meu14-GFP/Psy1-GFP at the end of Meiosis II (arrowheads in Figure S6B), which suggests that Meu14DD-GFP degradation was at least partly inhibited by the substitutions in the destruction box. We also found that the diameter of the FSM at maximum size was about 25% smaller on average in NP230 strains than in NP234 strains (Figure S6C), although the size of the mature spores were almost the same in both strains (data not shown). The results suggest that degradation of Meu14 is regulated by the ubiquitin-proteasome pathway, and that the ectopic expression of Meu14DD proteins caused abnormal FSM formation at the end of Meiosis II.

SUPPLEMENTARY REFERENCES

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Ectopic expression of Mug28-GFP suppressed the abnormal sporulation phenotype of mug28Δ cells. (A) Comparison of the spore viability between mug28+ (AS6), mug28Δ (AS99), mug28+::mug28+-GFP (AS108) and Mug28-GFP (AS164) strains indicates the almost complete suppression of the reduced spore viability in mug28Δ (AS99) cells. The data show the averages of three independent experiments with standard errors (error bars). At least 200 spores were measured for each strain. (B) Frequency of cells with normal and abnormal morphologies of these mug28+ (AS6), mug28Δ (AS99), mug28+::mug28+-GFP (AS108) and Mug28-GFP (AS164) strains at sporulation indicate the almost complete suppression of the appearance of abnormally shaped spores in mug28Δ (AS99) cells. (C) Typical fluorescence images of the control strain showing that GFP-tagged Mug28 in the mug28+::mug28+-GFP (AS108) strain displayed a similar localization as in the mug28+::mug28+-GFP (AS108) strain during meiosis. Fluorescence from Mug28-GFP was observed at meiosis I and II, after meiotic induction by nitrogen starvation. Fluorescence microscopy images of DNA (blue) and Mug28-GFP (green), together with their merged images. Bar, 10 µm.

Figure S2. DNA microarray analysis revealed the meiotic expression profiling of S. pombe genes in mug28Δ cells. (A) The h+/h- pat1-114 (JZ670) and pat1-114 mug28Δ (AS116) strains were induced to enter meiosis synchronously. The cells were collected at 0, 4, and 8 h time points and total RNAs were harvested for DNA microarray analysis as 2 color experiments (4/0 hour of JZ670, 8/0 hour of JZ670, 4/0 hour of AS116 and 8/0 hour of AS116). The time points of the collections are indicated by arrows. (B) Heat map analysis. (i) Hierarchical clustering of 3,616 genes is presented using the analytically available data. Rows and columns represent the analyzed genes and their expression profiles in JZ670 and AS116 strains at 4 and 8 h after nitrogen starvation. The log2 ratio of expression levels at each time point (4 and 8 h) relative to the levels at 0 h are color-coded as indicated at the bottom. (ii) Hierarchical clustering for the meiotically up-regulated genes that showed distinct expression patterns in mug28Δ cells compared to mug28+ cells. (ii) Hierarchical clustering for the meiotically down-regulated genes that showed distinct expression patterns in mug28Δ cells compared to mug28+ cells. (iv) Hierarchical clustering of 19 meu genes that were identified previously (Watanabe et al., 2001). (v) Hierarchical clustering of 6 genes whose products are associated with the 'expressed during meiosis' term in the Sz. pombe GeneDB (http://www.genedb.org/genedb/pombe/). (vi) Hierarchical
clustering of 9 genes whose products are associated with the 'ascospore-type prospore formation' term in the DB. (C) Bar graph indicates the expression levels of \textit{meu14}^+, \textit{meu6}^+, \textit{leu1}^+ and \textit{atb2}^+ genes; the y axis shows the fold-change relative to the 0 h time point. The \textit{leu1}^+ and \textit{atb2}^+ genes are shown as low level expression controls to compare the bar graph impression with that of heat map.

**Figure S3.** Microscopic visualization of Meu10-GFP and FSM formation. (A) Subcellular localization of Meu10-GFP in living NP250 ($h^{90}$ \textit{meu10}^+-GFP \textit{mCherry-psy1}^+; i) and NP251 ($h^{90}$ \textit{meu10}^+-GFP \textit{mCherry-psy1}^+ \textit{mug28}^+::\textit{ura4}^+; ii-iv) cells. Fluorescence signals from Meu10-GFP were observed during sporulation after meiotic induction by nitrogen starvation. Fluorescence microscopy images of DNA (blue), mCherry-Psy1 (red), and Meu10-GFP (green) are shown. White arrowheads denote Meu10-GFP localized to deformed mother ascospores displaying budded or snowman-like morphology. Bar, 10 µm. (B) FSM formation in \textit{psy1}^+-GFP strains (FY12104) ectopically expressing Meu14-9myc under various \textit{nmt} promoters. Psy1-GFP cells harboring pRM1, pRM41, or pRM81 plasmids (\textit{meu14}^+-9myc driven by the \textit{nmt}1, \textit{nmt}41 and \textit{nmt}81 promoters, respectively) were meiotically induced on sporulation plates without thiamine for ectopic expression at 30°C for 18 h. Psy1 localization in each strain was classified into three groups: normal (a), budded (b), and snowman-like (c). At least 250 spores were measured.

**Figure S4.** Abnormal phenotypes were observed in Psy1-GFP in \textit{mug28}Δ\textit{meu14}Δ double mutant cells. (A) GFP-Psy1 dynamics and nuclei in cells from meiosis II to sporulation are shown in wild type (YN68; i-ii), \textit{meu14}Δ (NP226; iii-v), \textit{mug28}Δ (AS117; vi-vii) and \textit{mug28}Δ\textit{meu14}Δ (NP230; viii-x) cells harboring \textit{psy1}^+-GFP. Cells were induced to enter meiosis at 28°C for 2 days, and then stained with Hoechst33342 (0.5 µg/ml) for nuclear visualization. Psy1-GFP (green), nucleus (DNA; blue) and their merged images are shown. Red or yellow arrowheads denote the deformed mother ascospores harboring budded or snowman-like patterns. Bar, 10 µm. (B) The histogram shows the percentage of normal and abnormal cells expressing Psy1-GFP in wild type (YN68), \textit{meu14}Δ (NP226), \textit{mug28}Δ (AS117) and \textit{mug28}Δ\textit{meu14}Δ (NP230) cells. Images are classified as class I (meiotic nuclei are fragmented), class II (FSMs are encapsulated into four nuclei), class III (Psy1-GFP show budded appearance), class IV (Psy1-GFP show snowman-like appearance) and class V (other phenotypes). At least 100 cells were counted.
Figure S5. Time-lapse images of FSM formation and/or the leading edge protein behavior in mug28\(^+\) (YN68, YOD50, SOP091w) cells at 28°C. GFP images that were captured every 6 min are presented for the YN68 (GFP-psy1\(^+\)) strain (A) and the YOD50 (meu14\(^+\)-GFP) strain (B). GFP images that were captured every 2.5 min are also shown for the SOP091w (meu14\(^+\)-GFP GFP-psy1\(^+\)) strain (C). Numbers indicated at the bottom of some photographs denote the time, in minutes, from the onset of FSM formation. Scale bars, 10 \(\mu\)m.

Figure S6. Delayed disappearance of Meu14 rings during meiosis is independent on the snowman-like phenotype of the mug28\(\Delta\) strain. (A) A comparison of FSM formation in Meu14-GFP (NP234: wild type) and Meu14DD-GFP (NP239: a mutant) cells harboring psy1\(^+\)-gfp. NP239 cells express Meu14DD-GFP with alanine substitutions at R135, L138, R148 and L151 residues located within the two putative destruction boxes (RxxL motif; see Materials and methods). Cells were meiotically induced on sprulation plates at 28°C for 24 h and stained with Hoechst33342 (0.5 \(\mu\)g/ml) for nuclear visualization. Typical images (DIC, DNA, Meu14-GFP/Psy1-GFP and the merged images) of meiotic Meu14-GFP (left panel) and Meu14DD-GFP (right panel) cells are shown. Bar, 10 \(\mu\)m. (B) Typical images of meiotic Meu14-GFP (NP234) and Meu14DD-GFP (NP239) are shown. Bar, 10 \(\mu\)m. (C) Comparison of the diameter of Meu14 rings between Meu14-GFP and Meu14DD-GFP strains. Three independent strains of Meu14-GFP (NP234, NP235 and NP236) and Meu14DD-GFP (NP239, NP240 and NP241) were collected and the Meu14 ring diameter was measured. A plot of the diameter of the Meu14 ring (y-axis), the independently measured strains (x-axis) and the average values (arrowhead) are shown. Inset shows a schematic presentation of the diameter of the Meu14 ring. Diameters of leading edges were measured using MetaVue 7.5.0.0 software (Molecular Devices, Downingtown, PA).

Figure S7. Typical images of TEM photographs of mug28\(^+\) (AS6) and mug28\(\Delta\) (AS99) spores that were used for the measurement of spore wall thickness. Spore wall thickness was measured at the positions indicated with white bars. Black scale bar, 0.5 \(\mu\)m.

Figure S8. Spore viability of Mug28-GFP (AS164), Mug28-RRM3\(\Delta\)-GFP (AS175), Mug28-3HA (AS197), and Mug28-RRM3\(\Delta\)-3HA (AS200) strains. Spore viability was measured by random spore analysis. At least 200 spores were measured.
Figure S9. No genetic interaction was observed between \textit{mug}28^{+} and \textit{sst}4^{+} genes during meiosis. (A) Subcellular localization of Sst4-mRFP in living AS214 (h^{90} sst4^{+}-mRFP mug28\Delta) cells. Fluorescence signals from Sst4-mRFP were observed at various stages of meiosis after meiotic induction by nitrogen starvation. DIC images are shown in the leftmost panels. DNA (blue), Sst4-mRFP (red) and the corresponding merged images are shown (B) Subcellular localization of Mug28-GFP in living AS217 (h^{90} mug28^{+}-GFP sst4\Delta) cells. Fluorescence signals from Mug28-GFP were observed at various stages of meiosis after meiotic induction by nitrogen starvation. DIC images are shown in the leftmost panels. DNA (blue), Mug28-GFP (green) and the corresponding merged images are shown. Bar, 10 \mu m. (C) Spore viability of wild type (AS6), mug28\Delta (AS99), sst4\Delta (MI368) and mug28\Delta sst4\Delta (AS213) strains. Spore viabilities were measured by random spore analysis. Bar graphs show the averages of three independent experiments with standard errors (error bars). At least 200 spores were measured.
Table S1. Yeast strains used in this study.

| Strain name | Genotype | Source/reference |
|-------------|----------|------------------|
| CD16-1      | h+/h- ade6-M210/ade6-M216 cyh1+/+/lys5-391 | C. Shimoda       |
| CD16-5      | h+/h- ade6-M210/ade6-M216 cyh1+/+/lys5-391 | C. Shimoda       |
| AS6         | h90 ade6-M210 leu1-32 ura4-D18          | This study       |
| AS99        | h90 ade6-M210 leu1-32 ura4-D18 mug28+::ura4+ | This study       |
| JZ670       | h/h ade6-M210/ade6-M216 leu1-32/pat1-114/pat1-114 | M. Yamamoto   |
| AS127       | h90 ade6-M210 leu1-32::sad1-mCherry-leu1+ ura4-D18 mug28+::mug28+-GFP-ura4+ | This study |
| YN68        | h90 leu1-32<<GFP-psy1+-leu1+ | This study       |
| AS117       | h90 ade6-M216 leu1-32<<GFP-psy1+-leu1+ ura4-D18 mug28+::ura4+ | This study       |
| AS122       | h/h ade6-M210/ade6-M216 leu1-32 ura4-D18/ura4-D18 pat1-114/pat1-114 mug28+::[mug28+-3HA-ura4+]/mug28+::[mug28+-3HA-ura4+] | This study |
| YDO50       | h90 leu1-32 ura4-D18 meu14+::meu14+-GFP spo3+-HA-LEU2+ | D. Okuzaki |
| YDO121      | h90 ade6-M216 his2 leu1-32 ura4-D18 meu14+::meu14+-GFP | D. Okuzaki |
| AS160       | h90 leu1-32 ura4-D18 mug28+::ura4+ meu14+::meu14+-GFP | This study |
| SOP091w     | h90 leu1-32 ura4-D18 leu1<<GFP-psy1+ meu14+::meu14+-GFP | A. Ohtaka |
| AS161       | h90 ade6-M216 leu1-32::psy1+-GFP-leu1+ ura4-D18 mug28+::ura4+ meu14+::meu14+-GFP | This study |
| AS164       | h90 ade6-M210 leu1-32::mug28+-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS172       | h90 ade6-M210 leu1-32::mug28+-RRM1A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS173       | h90 ade6-M210 leu1-32::mug28+-RRM2A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS174       | h90 ade6-M210 leu1-32::mug28+-RRM2A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS175       | h90 ade6-M210 leu1-32::mug28+-RRM1A-RMM3A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS176       | h90 ade6-M210 leu1-32::mug28+-RRM3A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS177       | h90 ade6-M210 leu1-32::mug28+-RRM1A-RMM2A-GFP-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS185       | h90 ade6-M210 leu1-32::mug28+-3HA-leu1+ ura4-D18 mug28+::ura4+ | This study |
| FY13203     | h90 ade6<<YFP-psy1+ leu1-32 | NBRP |
| AS197       | h90 ade6<<YFP-psy1+ leu1-32::mug28+-3HA-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS198       | h90 ade6<<YFP-psy1+ leu1-32::mug28+-3HA-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS199       | h90 ade6<<YFP-psy1+ leu1-32::mug28+-RRM2A-3HA-leu1+ ura4-D18 mug28+::ura4+ | This study |
| AS200 | $h^{00}$ ade6<<YFP-psyl+ leu1-32::mug28^-RRM3Δ3HA-leu1^+ ura4-D18 mug28^+::ura4^+ | This study |
|-------|------------------------------------------------------------------|-----------|
| AS201 | $h^{00}$ ade6<<YFP-psyl+ leu1-32::mug28^-RRM2ΔRRM3Δ3HA-leu1^+ ura4-D18 mug28^+::ura4^+ | This study |
| AS202 | $h^{00}$ ade6<<YFP-psyl+ leu1-32::mug28^-RRM1ΔRRM3Δ3HA-leu1^+ ura4-D18 mug28^+::ura4^+ | This study |
| AS203 | $h^{00}$ ade6<<YFP-psyl+ leu1-32::mug28^-RRM1ΔRRM2Δ3HA-leu1^+ ura4-D18 mug28^+::ura4^+ | This study |
| AS116 | $h^+/h^-$ ade6-M210/ade6-M216 leu1-32/leu1-32 pat1-114/pat1-114 mug28^+::ura4^+/mug28^+::ura4^+ | This study |
| FY12104 | $h^{00}$ ade6<<GFP-psyl+ leu1-32 | NBRP |
| NP234 | $h^{00}$ ade6<<psy1^-GFP meu14^-GFP leu1-32 ura4-D18 | This study |
| NP235 | the parent strain of NP234 strain | This study |
| NP236 | the parent strain of NP234 strain | This study |
| NP239 | $h^{00}$ ade6<<psy1^-GFP meu14DD-GFP leu1-32 ura4-D18 | This study |
| NP240 | the parent strain of NP239 strain | This study |
| NP241 | the parent strain of NP239 strain | This study |
| NP226 | $h^{00}$ ade6-M216 ura4-D18 leu1<<GFP-psyl^+ meu14^+::ura4^+ | This study |
| NP230 | $h^{00}$ ade6-M216 ura4-D18 leu1<<GFP-psyl^+ meu14^-::ura4^- mug28^+::ura4^+ | This study |
| TTM101 | $h^-$ ade6-M210 ura4-D18 leu1-32 meu10^-GFP | T. Tougan |
| NP250 | $h^{00}$ ade6-M210 ura4-D18 leu1-32::mCherry-psyl^+-leu1^+ meu10^-GFP | This study |
| NP251 | $h^{00}$ ade6-M210 ura4-D18 leu1-32::mCherry-psyl^+-leu1^+ meu10^-GFP mug28^-::ura4^- | This study |
| NP252 | $h^{00}$ ade6-M210 leu1-32::mug28F466A-GFP-leu1^+ ura4-D18 | This study |
| AS181 | $h^{00}$ ade6-M210 leu1-32::mCherry-psyl^+-leu1^+ ura4-D18 mug28^+::mug28-GFP-ura4^+ | This study |

NBRP: National BioResource Project [http://yeast.lab.nig.ac.jp/nig/]
Table S2. Oligonucleotide primers used in this study

| Primer                      | Sequence                                                                 |
|-----------------------------|--------------------------------------------------------------------------|
| mug28-5’Fw-KpnI             | 5’-GCGGTACCGAGACAAATATGTAATAAAAGCTGTGTAGT-3’                            |
| mug28-5’Rv-Hol              | 5’-GCCTCGAGGTGTAGTGTCTAGTGTGGTAAATTACAAAT-3’                             |
| mug28-3’Fw-Hol-stop-PstI    | 5’-GCCTCGAGTACAATGCCGATAAAAGCAAGAATATGCTCAAAGGACATAA-3’                 |
| mug28-3’Rv-Sacl            | 5’-GCGAGCTTCTTACTCTTTTGGTACTGGTGCTCTGTGAC-3’                            |
| mug28-5’Fw-Ascl-SalI       | 5’-GCGGCGCGCGCCCGTGACGATGATTGCACCTGGAAATTTTC-3’                          |
| mug28-3’Rv-NotI(non-stop codon) | 5’-GCGCGCGCGCCCTTGGTATAACTGATGGTTGAAGG-3’                               |
| 5’UTR-Fw-KpnI               | 5’-CGGGGTACCGCTCTACTATAAAAGACATCTGGTG-3’                                |
| inORF-plus-SalI-Rv          | 5’-ACGCGTCGACCTTTTCTGAAAGGTTTTTTCAAGTGCAATCAT-3’                        |
| inORF-plus-SalI-Fw          | 5’-ACGCGTCGACAAACAAAAGGACACTAAAGGATGAGGAATTT-3’                         |
| inORF-BamHI-Rv              | 5’-GTAAGATGGATTCCTTACATACGGCAATGCGTGAAATTTC-3’                          |
| RRM1Δ-Spel-Rv               | 5’-GACTAGTGCTCTCGCTTGGTGTCGAC-3’                                       |
| RRM2A-BamHI-Rv              | 5’-GCAGAATCCGTCGAACAAAAATGGGATCTGTTCTTGAAGAC-3’                         |
| RRM3Δ-NotI-Rv               | 5’-GCAGCGCGCCCGCCGCTTGAGGTGTGTTGTTGCG-3’                                |
| RRM3O-SalI-Fw               | 5’-GCAGTCGACACTGTTGAGAGATGATAAAAGGATTGG-3’                             |
| RRM1O-NotI-Rv               | 5’-GCAGCGCGCCCGCCAGGGTTGTCGACAAAAATGGG-3’                               |
| RRM2O-SalI-Fw               | 5’-GCAGTCGACACTGTTGAGAGATGATAAAAGGATTGG-3’                             |
| RRM3Δ-NotI-Rv               | 5’-GCAGCGCGCCCGCCAGGGTTGTCGACAAAAATGGG-3’                               |
| D1-coding                   | 5’-CACGAGACAGATGCAACAAGGACGATCTCAAGACATTG-3’                            |
| D2-coding                   | 5’-GTGACGGAGAAAGCAGACCATCGTCCTCTCAACATCTAC-3’                           |
| D1-noncoding                | 5’-GGCTCTGTGTCATGTCGTGAGGTTGCGAT-3’                                     |
| D2-noncoding                | 5’-GGACGGATCTGGCTTTTTCGTCAATGCTTCAATGCT-3’                              |

Restriction enzyme sites are underlined. The codons used for alanine substitutions are denoted by bold italics.
### Table S3. Microarray data used to draw Figure S2 were presented below.

(A) Microarray data for Fig. S2C.

|                | Fold Change |
|----------------|-------------|
|                | *mug28* 4h | *mug28* 8h | *mug28Δ* 4h | *mug28Δ* 8h |
| *meu14*        | 80.3       | 9.1        | 122.7       | 6.6         |
| *meu6*         | 7.7        | 127.2      | 230.4       | 356.0       |
| *leu1*         | -2.4       | -1.2       | -2.3        | -2.8        |
| *atb2*         | 3.3        | -1.2       | 2.5         | -1.1        |

(B) Microarray data for Fig. S2B-ii.

|                | Fold Change |
|----------------|-------------|
|                | *mug28* 4h | *mug28* 8h | *mug28Δ* 4h | *mug28Δ* 8h |
| SPCC1919.05    | 6.8        | 1.0        | 5.3         | -3.8        |
| SPBP23A10.03C  | 3.2        | -1.2       | 4.1         | -4.7        |
| *rad60*        | 45.8       | 1.3        | 36.8        | 3.7         |
| *chp2*         | 1.3        | -2.8       | 4.3         | -4.2        |
| SPCC1529.01    | 7.5        | -1.4       | 1.5         | -1.9        |
| SPCC645.11C    | 7.1        | 1.5        | 8.9         | -5.0        |
| SPBC30D10.07C  | 6.5        | -1.1       | 6.5         | -3.5        |
| *sut1*         | 64.9       | 4.3        | 69.7        | -1.0        |
| *meu6*         | 7.7        | 127.2      | 230.4       | 356.0       |
| *ght3*         | 16.7       | 1.3        | 7.3         | -2.7        |

(C) Microarray data for Fig. S2B-iii.

|                | Fold Change |
|----------------|-------------|
|                | *mug28* 4h | *mug28* 8h | *mug28Δ* 4h | *mug28Δ* 8h |
| *aes1*         | -19.5      | -1.4       | -100.0      | -38.4       |
| *ade8*         | -1.4       | 1.5        | -6.0        | 1.3         |
| SPAC3A11.10C   | -3.9       | 1.8        | -9.4        | -1.4        |
| SPAC3H8.07C    | -5.6       | 1.2        | -4.0        | 3.2         |
| *nog1*         | -100.0     | -2.0       | -8.0        | -2.0        |
| SPAC27F1.05C   | -3.0       | -1.2       | -23.4       | -6.5        |
| *rad50*        | -12.6      | -4.5       | -2.8        | -8.1        |
| SPBC14C8.09C   | -6.0       | 1.0        | -3.7        | 3.1         |
(D) Microarray data for Fig. S2B-iv.

|        | Fold Change |        |        |        |        |
|--------|-------------|--------|--------|--------|--------|
|        | mug28+ 4h   | mug28+ 8h | mug28Δ 4h | mug28Δ 8h |
| meu14* | 80.3        | 9.1    | 122.7   | 6.6    |
| meu24* | 28.5        | 143.8  | 18.5    | 38.5   |
| meu17* | 399.3       | 77.6   | 413.7   | 47.9   |
| meu9*  | 5.7         | 11.4   | 9.9     | 15.6   |
| meu8+  | -2.0        | -1.2   | -7.6    | -4.4   |
| meu5*  | 30.4        | 108.3  | 61.4    | 163.0  |
| meu29* | -1.4        | 2.3    | -1.7    | 1.8    |
| meu7*  | 2301.5      | 851.9  | 1095.6  | 434.3  |
| meu18* | 31.1        | 2.3    | 14.9    | 5.2    |
| meu25* | 102.6       | 42.8   | 166.0   | 20.8   |
| meu12* | 75.6        | 13.3   | 74.9    | 16.7   |
| meu28* | 45.7        | 9.2    | 46.9    | 5.9    |
| meu21* | 37.8        | 2.3    | 36.4    | 2.8    |
| meu5*  | 340.4       | 13.8   | 236.6   | 4.2    |
| meu30* | -1.3        | -1.9   | -1.0    | -3.0   |
| meu6*  | 7.7         | 127.2  | 230.4   | 356.0  |
| meu10* | 1656.4      | 91.7   | 710.5   | 75.2   |
| meu4*  | 912.6       | 1445.6 | 634.1   | 460.6  |
| meu27* | 67.5        | 74.6   | 95.8    | 89.8   |

(E) Microarray data for Fig. S2B-v.

|        | Fold Change |        |        |        |        |
|--------|-------------|--------|--------|--------|--------|
|        | mug28+ 4h   | mug28+ 8h | mug28Δ 4h | mug28Δ 8h |
| mcp4*  | 52.0        | 9.3    | 76.7    | 9.6    |
| mcp1*  | 15.2        | 2.3    | 7.8     | 3.1    |
| mcp*   | 3.1         | 1.8    | 5.4     | 2.5    |
| cam2*  | 2.2         | 1.1    | 2.5     | 1.3    |
| spn5*  | 45.7        | 9.2    | 46.9    | 5.9    |
| mcp5*  | 5.5         | -2.4   | 7.0     | -3.6   |
(F) Microarray data for Fig. S2B-vi.

|                | Fold Change          |
|----------------|----------------------|
|                | mug28+ 4h | mug28+ 8h | mug28Δ 4h | mug28Δ 8h |
| spo3⁺          | 161.0     | 12.5      | 121.9     | 20.4      |
| cta4⁺          | 1.3       | -1.9      | 1.1       | -2.2      |
| spo14⁺         | -2.0      | 1.2       | -2.4      | -1.1      |
| meu14⁺         | 80.3      | 9.1       | 122.7     | 6.6       |
| spo20⁺         | -1.8      | -1.3      | -1.9      | -1.4      |
| psy1⁺          | 5.5       | 2.1       | 6.3       | 2.5       |
| cam2⁺          | 2.2       | 1.1       | 2.5       | 1.3       |
| ypt7⁺          | -1.2      | 1.1       | -1.1      | 1.0       |
| sec9⁺          | 3.2       | -1.4      | 4.3       | -1.3      |

(G) Microarray data for Fig. S2B-vii.

|                | Fold Change          |
|----------------|----------------------|
|                | mug28+ 4h | mug28+ 8h | mug28Δ 4h | mug28Δ 8h |
| bgs2⁺          | 37.8      | 2.3       | 36.4      | 2.8       |
| cam2⁺          | 2.2       | 1.1       | 2.5       | 1.3       |
| chs1⁺          | 2.3       | -2.4      | 2.5       | ND        |
| crp79⁺(meu5⁺)  | 340.4     | 13.8      | 236.6     | 4.2       |
| acp1⁺          | -3.7      | -1.0      | -4.2      | -1.0      |
| acp2⁺          | 1.3       | -1.4      | 1.3       | -2.0      |
| myo1⁺          | 1.5       | 1.4       | 1.1       | -1.0      |
| sst4⁺/vsp27⁺   | 6.2       | 2.5       | 2.6       | 1.4       |
| spo20⁺         | -1.8      | -1.3      | -1.9      | -1.4      |
Figure S1. Shigehisa et al.

A

![Bar chart showing spore viability](chart.png)

- **mug28**
- **mug28Δ**
- **mug28**:mug28**:gfp
- **Mug28-GFP**

- 80.2% ± 1.5%
- 77.0% ± 1.2%
- 97.2% ± 0.8%

B

![Bar chart showing frequency of cells](chart.png)

- **mug28**
- **mug28Δ**
- **mug28**:mug28**:gfp
- **Mug28-GFP**

- Normal morphology
- Abnormal morphology

C

**mug28**:mug28**:gfp

- DNA
- GFP
- Merged

- DNA
- GFP
- Merged

**Mug28-GFP**

- DNA
- GFP
- Merged

- DNA
- GFP
- Merged
Figure S3. Shigezasa et al.

A

|        | DNA      | mCherry-Psy1 | Meu10-GFP |
|--------|----------|--------------|-----------|
| wt     |          |              |           |
| (i)    |          |              |           |
| (ii)   |          |              |           |
| (iii)  |          |              |           |
| (iv)   |          |              |           |
| mug28Δ |          |              |           |

B

| Psy1-GFP | nmt1 meu14*-9myc | nmt41 meu14*-9myc | nmt81 meu14*-9myc |
|----------|------------------|-------------------|-------------------|
| a) Normal| 98.7 % ± 1.1 %   | 97.9 % ± 2.1 %    | 97.6 % ± 2.2 %    |
| b) Abnormal | 1.1 % ± 1.0 %   | 2.1 % ± 2.1 %    | 1.6 % ± 2.2 %    |
| c) Abnormal | 0.2 %± 0.3 %   | 0.0 %             | 1.8 %± 1.4 %    |
Figure S5. Shigehisa et al

A. mug28+ GFP-Psy1

B. mug28+ Meu14-GFP

C. mug28+ Meu14-GFP GFP-Psy1
Figure S6. Shigehisa A, et al.

A

B

C

Diameter of leading edge

|          | Meu14-GFP | Meu14DD-GFP |
|----------|-----------|-------------|
| average  | 2.35      | 2.46        |
| SD       | 0.28      | 0.35        |
| n        | 185       | 191         |

Diameter (μm)
Figure S7. Shigehisa et al

$mug28^+$

$mug28\Delta$
Figure S9. Shigeisna et al.

A. 

|          | DIC | DNA | Sst4-mRFP | Merge |
|----------|-----|-----|-----------|-------|
| Vegetative |     |     |           |       |
| Horsetail |     |     |           |       |
| Meiosis I  |     |     |           |       |
| Meiosis II |     |     |           |       |

B. 

|          | DIC | DNA | Mug28-GFP | Merge |
|----------|-----|-----|-----------|-------|
| Vegetative |     |     |           |       |
| Horsetail |     |     |           |       |
| Meiosis I  |     |     |           |       |
| Meiosis II |     |     |           |       |

C. 

|       | Score viability (%) |
|-------|---------------------|
| wild type |             |
| mug28Δ    |             |
| sst4Δ     |             |
| mug28Δ sst4Δ |             |