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Sim3 shares some common roles with the histone chaperone Asf1 in fission yeast

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Running title: Relationship between Sim3 and Asf1

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ABSTRACT

An H3/H4 histone chaperone, Asf1, plays an essential role in maintaining genomic stability in many species, including fission yeast. Here, we showed that overexpression of a CENP-A chaperone Sim3 suppressed the temperature sensitive phenotype of *asf1-33* and *asf1-30* mutants and the defect in chromatin structure, and prevented the accumulation of DNA damage in *asf1-33* mutants at high temperatures. Furthermore, *asf1-33* and Δ*sim3* were synthetic lethal. Consistent with this, shutdown of *sim3* expression in *asf1-33* Δ*sim3* double mutants that contained extragenic *sim3* resulted in growth retardation. In addition, the Δ*sim3* mutant displayed sensitivity to thiabendazol and hydroxyurea, which suggests that Sim3 plays a general role in maintaining chromatin structure. Our results suggest a possibility that Sim3 functions as a histone chaperone.

1. Introduction

Histone chaperones mediate changes in chromatin structure through the deposition and eviction of histones [1]. The structure of chromatin is altered in response to cellular processes that occur in DNA, such as DNA replication, transcription, recombination, and DNA repair. For example, when a gene is expressed in response to certain signals, histone proteins are evicted from the promoter region by histone chaperones to disassemble chromatin for transcription [2]. In addition, when DNA damage is repaired, histone proteins are temporarily evicted from the damaged site by histone chaperones to enable repair factors to function. Then, histone proteins are deposited back onto the repaired sites by histone chaperones after the completion of repair [3].

Histone chaperones are categorized into several groups by their preference for histone proteins: Asf1 (anti-silencing function 1) [4], CAF1 (chromatin assembly factor 1) [5], and HIRA (histone interacting protein A) [5] are considered to be histone H3/H4 chaperones, while Nap1 (nucleosome assembly protein 1) [5] (Eitoku, Sato, Senda, & Horikoshi, 2008)is a histone H2A/H2B chaperone. Histone variants are assembled or disassembled into chromatin by special histone chaperones that are specific for each histone variant. For example, Asf1 generally deposits (and evicts) histone H3/H4 onto chromatin. However, Asf1 does not bind to histone H3 variant CENP-A; instead, HJURP incorporates CENP-A onto centromeres in human cells [6].

Chromosome segregation is a pivotal process required for the transmission of genetic material to daughter cells. When chromosomes segregate, spindle microtubules emanating from SPB (spindle pole bodies) or centrosomes attach to the kinetochores of chromosomes. The kinetochore is composed of large protein complexes and centromeric DNA [7], and its formation is specified by CENP-A [8]. CENP-A is specifically localized to the center of the centromere region, while histone H3 is distributed to all other chromosomal regions. The loading of CENP-A onto centromeres requires many factors including Sim3 and Scm3. Sim3 was identified as a factor that functions in the maintenance of silencing at the center centromeric regions in fission yeast *Schizosaccharomyces pombe* [9]. Sim3 binds to CENP-A ^{Cnp1}, and transfers it to a CENP-A ^{Cnp1} receptor, Scm3 [10], on centromere

chromatin. This results in the incorporation of CENP-A ^{Cnp1} into the center region of centromeres in *S. pombe*.

Previously, we reported that an *asf1 ts* mutant (*asf1-33*) showed defects in bulk chromatin structure and displayed elevated levels of DNA damage, which was associated with activation of the DNA damage checkpoint [11]. Based on these observations, we suggested that fission yeast Asf1 is essential for the maintenance of genomic stability. In addition, we found that overexpression of CENP-A ^{Cnp1} histone chaperone *sim3* suppressed the temperature sensitive phenotype of the *asf1-33* mutant. Here, we showed that overexpression of *sim3* also suppressed the defects in chromatin structure and prevented the accumulation of DNA damage in the *asf1-33* mutant. Furthermore, our results reveal that *asf1*-33 Δ*sim3* double mutants were synthetically lethal. In summary, our results indicate that CENP-A histone chaperone Sim3 shares some functional characteristics with Asf1 as a histone H3/H4 chaperone in fission yeast.

2. Materials and methods

2.1. Fission yeast media and general manipulations

The yeast strains examined in this study are all listed in Table 1. Each strain was cultured in YES medium (0.5% yeast extract, 3% glucose, 225 mg/liter adenine, histidine, leucine, uracil, and lysine hydrochloride) or EMM medium [12]. Nitrogen-free EMM medium was used to mate h^+ and h^- strains. Standard methods used for *S. pombe* were described previously [12].

2.2. Detection of Rad22-GFP foci

Rad22-GFP foci in *asf1-33* mutants harboring pREP41 or pREP41-*sim3* $^+$ were observed by a confocal laser scanning microscope (Leica TCS SP-5, Leica Microsystems, Tokyo). The number of Rad22-GFP positive cells was calculated by counting 100 cells.

2.3. Micrococcal nuclease assay

Micrococcal nuclease assay was performed as described previously [13]. Cells were cultured in EMM at 36°C for 6 h. After incubation, cells were collected by centrifugation. Then, spheroplasts were prepared with

Zymolyase 100T. Chromatin DNA was digested with MNase. Digested chromatin DNA was resolved on a 1.2% agarose gel and was visualized by ethidium bromide staining.

2.4. Construction of a sim3 gene deletion strain and pREP81-sim3

Gene deletion of *sim3* was carried out using a PCR-based method [14]. The kanMX6 module was amplified using pFA6a-kanMX6 as a template together with the pFA6a F and pFA6a R primers (Table S1). Fragments approximately 500 bp in length were amplified using sim3d1, sim3d2, sim3d3, and sim3d4 primers, which contained homologous sequences corresponding to the 5’ and 3’ regions of *sim3* that were attached to the ends of the kanMX6 module. The resulting fragments were introduced into *S. pombe* cells. G418 resistant colonies were selected on YES plates containing G418 (100 µg/ml). Colony PCR (using the primers chkHR12-42 and sim3 check) was performed to confirm the construction of a Δ*sim3* strain. For the construction of pREP81-sim3, the *sim3* gene was cloned into pREP81 using a gap-repair cloning method [15]. The ORF region of *sim3* containing a sequence homologous to pREP81 was amplified by PCR. This fragment, together with BamHI digested pREP81, was introduced into PR110 (h^- *leu1-32 ura4-D18*), and transformants were selected on EMM-Leu. The plasmids were extracted from the transformants and introduced into *Escherichia coli* DH5✔ to amplify the plasmids. The correct construction of the plasmid was confirmed by sequencing using the primers Pnmt1 80 bp F and Tnmt1 80 bp R.
An *asf1-33* Δ*sim3* strain harboring pREP41-*sim3* or pREP81-*sim3* was constructed by chromosomal deletion of *sim3* from an *asf1-33* strain harboring pREP41-*sim3* or pREP81-*sim3*. Proper deletion of *sim3* was confirmed by PCR as described above.

3. **Results**

3.1. Overexpression of sim3 suppressed the defect in chromatin structure and prevented the accumulation of DNA damage in the asf1-33 mutant

We previously reported that overexpression of *sim3* suppressed the temperature sensitive phenotype of the *asf1-33* mutant in *S. pombe* [11]. In

addition to the role of Sim3 as a CENP-A ^{Cnp1} histone chaperone that transfers CENP-A ^{Cnp1} protein to the center region of centromeres (*cnt*, *imr*), it has also been reported that Sim3 has an ability to bind histone H3 [17]. Therefore, we hypothesized that Sim3 might be able to replace Asf1 as a H3/H4 histone chaperone. To test this idea, we used an MNase assay to examine the bulk chromatin structure of an *asf1-33* mutant that overexpressed *sim3*. In the *asf1-33* mutant harboring the control vector, adding increasing amounts of MNase showed that the bulk chromatin was digested into small units of chromatin. By contrast, the chromatin from the *asf1-33* mutant that overexpressed *sim3* was resistant to MNase digestion (Fig.1A), which indicates that *sim3* complemented the function of *asf1* in maintaining chromatin structure.

To examine further the relationship between *asf1* and *sim3*, the heterochromatin structure at the outer repeats of centromeres in the *asf1-33* mutant overexpressing *sim3* was analyzed. Heterochromatin exhibits a condensed chromatin structure in which gene expression is repressed [18]. By monitoring the *ura4*$^{+}$ marker gene inserted at the outer repeats of a centromere, we previously reported that heterochromatin structure at the outer centromeric repeats was disrupted in the *asf1-33* mutant [11]. 5-FOA sensitivity was observed as a result of increasing the transcriptional level of the *ura4*$^{+}$ gene at the outer repeats of the centromere in the *asf1-33* mutant. To examine whether *sim3* would suppress the defect in heterochromatin structure in the *asf1-33* mutant, we examined the expression of the *ura4*$^{+}$ gene inserted at the outer repeats of a centromere. We found that overexpression of *sim3* suppressed the 5-FOA sensitivity of the *asf1-33* mutant (Fig.1B), and likely its defect in heterochromatin structure.

We previously observed that the *asf1-33* mutant accumulates DNA damage that results in the loss of viability at 36°C [11]. We predicted that the suppression of temperature sensitivity in the *asf1-33* mutant by *sim3* overexpression was attributable to reduced levels of DNA damage. To determine whether the levels of DNA damage were reduced in the *asf1-33* mutant that overexpressed *sim3*, we analyzed the presence of Rad22-GFP foci in these cells. Rad22 protein involves in DNA repair mediated by homologous recombination. When DNA is damaged, this protein accumulates at the sites of DNA damages and forms foci. Overexpression of *sim3*

decreased the number of Rad22-GFP foci from about 19% to 6% in the *asf1-33* mutant at 36°C (Fig. 1C). Therefore, the levels of DNA damage in the *asf1-33* mutant were clearly reduced by *sim3* overexpression. Together, these results suggest that Sim3 can act as a substitute for Asf1 in *S. pombe*.

3.2. Overexpression of sim3 suppressed the temperature sensitivity and chromatin defects of the asf1-30 mutant

We previously reported that the mutant Asf1 protein (Asf1-30), which is encoded by *asf1-30,* was degraded by the ubiquitin-proteasome system at higher temperatures [19]. The mutation sites of *asf1-30* differ from *asf1-33,* and it was found that *asf1-30* displayed a higher degree of temperature sensitivity because of the absence of any detectable Asf1 protein at higher temperatures [19]. To test whether *sim3* can suppress the defects of the *asf1-30* mutant, we examined the temperature sensitivity of an *asf1-30* mutant strain that overexpressed *sim3*. While the *asf1-30* mutant did not grow at temperatures higher than 32°C, *sim3* expression clearly restored the growth of the *asf1-30* mutant at higher temperatures (Fig. 2A). Repressing *sim3* expression by the addition of thiamine in the *asf1-30* mutant harboring pREP41-sim3 again resulted in temperature sensitivity. These results indicate that *sim3* can replace the function of Asf1 even when Asf1 function is severely impaired.

To exclude the possibilities that overexpressed *sim3* might change localization or a protein level of Asf1-30 (or Asf1-33), we observed the localization of Asf1 proteins by indirect immunofluorescence microscope and protein levels by western blotting. The results showed that overexpression of *sim3* did not change the localization and protein levels of Asf1-33 and Asf1-30 both at 26 ^{o}C and 36°C (Supplementary Fig. 1A and 1B). Particularly, a lower protein level of Asf1-30 at higher temperature was not recovered even when *sim3* was overexpressed. These results indicate that up-regulation of Asf1 proteins or change of the localization was not a reason for suppression of temperature sensitivity of *asf1-30* and *asf1-33* mutants by *sim3*.

We next examined the structure of heterochromatin in the *asf1-30* mutant that overexpressed *sim3*. The *ura4* gene in the *otr1* locus is shut off in wild-type cells and is thus not sensitive to 5-FOA. The *asf1-30* mutation

restored 5-FOA sensitivity due to the reversion of *ura4* gene expression at 30°C (Fig. 2B). However, it is not clear that overexpression of *sim3* can suppress the 5-FOA sensitivity of the *asf1-30* mutant, because it is difficult to separate the effect of overexpression of *sim3* on the temperature sensitivity and the 5-FOA sensitivity in the *asf1-30* mutant (Fig. 2B).

Cellular morphology and phloxin B staining of *asf1-30* cells expressing *sim3* was also assessed (Fig. 2C). We found that the number of stained and elongated cells were reduced in *asf1-30* cells by the overexpression of *sim3,* which supports the idea that *sim3* has a suppressive effect on *asf1-30* cells.

3.4. asf1-33 Δsim3 double mutants display synthetic lethality

To further investigate the genetic relationship between *asf1* and *sim3*, we tried to construct an *asf1-33 Δsim3* double mutant. Strain TYP48 (h^- *asf1-33*-13myc-hphMX6) was crossed with KT146 (h^+ *sim3*::kanMX6), and their tetrads were analyzed. Although 19 sets of tetrads were dissected, we were unable to isolate any double mutants that were resistant to both G418 and Hygromycin B (Fig. 3A). We also performed random spore analysis to isolate double mutants, but no *asf1 Δsim3* double mutants were obtained (data not shown).

Because *asf1-33 Δsim3* double mutants display synthetic lethality, we constructed an *asf1-33* and *Δsim3* strain that harbored either the plasmid pREP41-*sim3* or pREP81-*sim3*. The expression of *sim3* gene was regulated by the *nmt41* or *nmt81* promoter in these double mutants, and the addition of thiamine partially and completely repressed *sim3* gene expression in pREP41(*nmt41*) and pREP81(*nmt81*), respectively. Without thiamine, an *asf1-33 Δsim3* strain that harbored pREP41-*sim3* or pREP81-*sim3* grew well at 34°C. However, the addition of thiamine to the same cells resulted in a growth defect (Fig. 3B). The *asf1-33 Δsim3* strain harboring pREP81-sim3 exhibited a more severe phenotype than the strain harboring pREP41-sim3, which indicated that even in the presence of thiamine, strains containing pREP41-sim3 display residual levels of *sim3*. Growth defect was observed in the *asf1-33 Δsim3* strain harboring pREP81-*sim3* at 34°C when thiamine was added. Together, these results indicate that *sim3* is required for the growth of the *asf1-33* mutant.

3.5. Deletion of sim3 results in TBZ and HU sensitivity

We next examined the drug sensitivity of Δ*sim3* cells. We found that Δ*sim3* cells displayed greater sensitivity to a spindle poison, TBZ (thiabendazole), at all temperatures, and to a DNA replication inhibitor, HU (hydroxyurea), at higher temperatures than wild-type cells (Fig. 4). Because Sim3 is a centromere chromatin assembly factor and *sim3* mutant cells show defects in chromosome segregation [1], it was a reasonable prediction that Δ*sim3* cells might be sensitive to TBZ. The HU sensitivity of Δ*sim3* cells suggested that there might be defects in S phase progression. However, FACS analysis did not reveal a delay in S phase progression in Δ*sim3* cells (data not shown). The growth defect in Δ*sim3* cells appeared to be more pronounced at lower temperatures (26°C) than at higher temperatures (30°C or higher). Therefore, the HU sensitivity of Δ*sim3* cells at higher temperatures might not cause severe defects. In contrast to TBZ and HU, Δ*sim3* cells did not show any sensitivity to a DNA damaging agent, MMS (methyl methanesulfonate). This is in contrast with our previous results showing that the *asf1-33* mutant displayed sensitivity to MMS, but not to HU [11].

4. Discussion

Asf1 is a commonly found histone chaperone that predominantly interacts with H3/H4 in many eukaryotes [1, 4]. Our analysis of *asf1* mutants in *S. pombe* revealed that *asf1* is essential for growth, and that its temperature sensitive mutation resulted in the accumulation of DNA damage and DNA breaks that induced DNA damage checkpoints at restricted temperatures [11]. A gene that encodes for a NASP type protein, *sim3*, was isolated as a high copy suppressor of the temperature sensitive *asf1-33* mutant in *S. pombe*. Sim3 was originally identified as a factor that interacts with CENP-A^{Cnp1}[17], but our analysis suggested that Sim3 plays a role as a H3/H4. It is interesting to note that the amino acid sequence and protein structure of Asf1 and Sim3 bear no similarity [17], despite their similar functions.

We showed that the overexpression of *sim3* suppressed the defect in bulk chromatin structure (Fig.1A) and reduced the accumulation of DNA damage

in the *asf1-33* mutant (Fig.1C). In addition, the silencing defect at the outer centromeric repeats of the *asf1-30* and *asf1-33* mutants was also suppressed by overexpressing *sim3* (Fig.1B and 2B). Therefore, Sim3 shares some common roles with Asf1. Since it is reported that Sim3 binds to histone H3 in addition to CENP-A^{Cnp1} [17], our results support the idea that Sim3 can function as a H3/H4 histone chaperone. In addition, we failed to construct an *asf1-33* Δ*sim3* double mutant; in other words, *asf1-33* and Δ*sim3* is a synthetically lethal combination. We also found that the Δ*sim3* mutant affects silencing at the centromeric region [17], but grows normally at 30°C. When it was combined with the *asf1-33* mutant, *sim3* was required for viability, which suggests that *sim3* shares an important role with *asf1*. Since our previous analysis indicated that mutated Asf1-33 proteins were unable to bind histone H3, the ability of the Sim3 protein to bind histone H3 might be required for the survival of the *asf1-33* mutant.

In addition to its histone H3 chaperone functions, Asf1 also plays a role in maintaining heterochromatin structure at the centromere. Heterochromatin structure was impaired in the *asf1* mutants (either *asf1-30* or *asf1-33*), but was then suppressed by Sim3 overexpression. The role of Sim3 was reported to be restricted to the center of the centromere region, but it seems that Sim3 may potentially act as a silencing maintenance factor at the outer centromeric repeats as well. Sim3 can substitute for Asf1 by incorporating (or removing) histone proteins onto chromatin. We found that the deletion of *sim3* resulted in the sensitivity to a spindle poison, TBZ, which is consistent with previous results by Pidoux *et al* (Pidoux, Richardson, & Allshire, 2003). In addition, Δ*sim3* cells displayed sensitivity to HU at 30°C or higher. However, cell cycle progression during S phase was not delayed in Δ*sim3* cells. Therefore, the effect of HU on Δ*sim3* cells was limited. As the deletion of *sim3* conferred sensitivity to HU and *asf1-33* conferred sensitivity to MMS, Sim3 likely plays a greater role in replication, while Asf1 may be more crucial in response to DNA damage. Different roles of Sim3 and Asf1 as a chaperone are envisaged from these drug sensitivity tests.

Scm3 (and its homolog, HJURP) has been shown to be involved in the incorporation of CENP-A to the center region of centromeres in several species, such as *S. pombe* [20], *S. cerevisiae* [21] and humans [6]. Scm3 localizes to the center region of centromeres and incorporates CENP-A into

centromeres. Although *sim3* overexpression suppressed the temperature sensitivity of the *asf1-33* mutant, *scm3* overexpression did not (data not shown). Likewise, overexpression of *asf1* did not suppress the temperature sensitivity of the *scm3* mutant (data not shown). Unlike Sim3, which is localized throughout the nucleus (Dunleavy et al., 2007), Scm3 localizes to and functions at only the centromere region. This specific localization of Scm3 may be one reason why we were unable to detect a genetic interaction between *scm3* and *asf1*.

In conclusion, our analysis of *sim3* and *asf1* indicates that Sim3 and Asf1 share some common roles as histone chaperones. Although Asf1 functions as the predominant histone chaperone in fission yeast, we suggest that Sim3 may also play a role as histone chaperone.

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Figure legends

Fig. 1. *sim3* overexpression suppressed the chromatin defects of the *asf1-33* mutant. (A) Overexpression of *sim3* suppressed the defects in bulk chromatin structure of the *asf1-33* mutant. SKP605-33 (h^{+} *leu1-32 ura4-D18 asf1-33*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3* was incubated in EMM (-Thi) at 36°C for 6 h. Cells were collected by centrifugation, and spheroplasts were prepared with Zymolyase 100T. MNase was added to each spheroplast to digest chromatin DNA. Digested chromatin DNA was resolved on a 1.2% agarose gel and was stained with ethidium bromide. (B) Defects in heterochromatin structure in the *asf1-33* mutant were suppressed by the overexpression of *sim3.* Logarithmically growing L972 (h^{-}), SKP551-6 (h^{+} *leu1-32 ura4-D18 otr1*::$ura4^{+}$) harboring pREP41,and SKP593-34 (h^{+} *leu1-32 ura4-D18 otr1*::$ura4^{+}$ *asf1-33*-13myc-kanMX6) cells harboring pREP41 or pREP41-*asf1* or pREP41-*sim3*were diluted 10-fold from 3x 10^{6} cells with sterilized water, and spotted onto EMM (+, -5FOA) . Plates were incubated at 26°C, 30°C, 32°C, 34°C, and 36°C for 5 days. (C) KT166 (h^{-} *rad22-GFP-*kan^{r} *asf1-33*-13myc-kanMX6 *leu1-32*) harboring pREP41 or pREP41-*sim3* were incubated in EMM (-Thi) at 36°C for 6 h. Rad22-GFP foci were observed with a confocal laser scanning microscope (Leica TCS SP-5). The rate of nuclear Rad22 foci was calculated by counting 100 cells.

Fig. 2. Overexpression of *sim3* suppressed the temperature sensitivity and defect in heterochromatin structure of the *asf1-30* mutant. (A) Overexpression of *sim3* suppressed the temperature sensitivity of the *asf1-30* mutant at 32°C, 34°C, and 36°C. Logarithmically growing L972, SKP620 (*h*[illegible] *leu1-32 asf1-30*-13myc-kanMX6) cells harboring pREP41 or pREP41-*sim3* were diluted 5-fold from 3x 10^{7} cells with sterilized water, and spotted onto EMM (+,-thi). Plates were incubated at 26°C, 32°C, 34°C, and 36°C for 3 days. (B) Overexpression of *sim3* suppressed the defect in heterochromatin structure of the *asf1-30* mutant at 30°C, 32°C, 34°C, and 36°C. Logarithmically growing L972 (h^{-}), SKP551-6 (h^{+} *leu1-32 ura4-D18 otr1*::$ura4^{+}$) harboring pREP41, and SKP593-35 (h^{+} *leu1-32 ura4-D18 otr1*::$ura4^{+}$ *asf1-30-13myc-*kan^{r}) cells harboring pREP41 or pREP41-*asf1* or pREP41-*sim3* were diluted 10-fold from 3x 10^{6} cells with sterilized water,

and spotted onto EMM (+,-5FOA) . Plates were incubated at 26°C, 32°C, 34°C, and 36°C for 5 days. (C) Overexpression of *sim3* suppressed the elongated cell shape and prevented cell death of the *asf1-30* mutant. SKP620 (h^- *leu1-32 asf1-30*-13myc-kanMX6) cells harboring pREP41 or pREP41-*sim3* were stained with phloxin B and observed by microscopy.

Fig. 3. *asf1-33* and Δ*sim3* double mutants were synthetically lethal. (A) TYP48 (h^- *asf1-33*-13myc-hphMX6 *leu1-32 ura4-D18*) was mated with KT146 (h^+ *sim3*::kanMX6 *leu1-32 ura4-D18*) on EMM (without nitrogen) for several days at 26°C. Then, tetrad analysis was carried out, and sporulated cells were cultured on YES (+G418 and Hygromycin to select *asf1-33* Δ*sim3* double mutants. However, no G418 and Hygromycin B resistant strains were obtained. (B) The *sim3* gene was disrupted in TYP48 (h^- *asf1-33*-13myc-hphMX6 *leu1-32 ura4-D18*) strains harboring pREP41-*sim3* or pREP81-*sim3*. Then, cultures of this strain (*asf1-33* Δ*sim3* mutant harboring pREP41-*sim3* or pREP81-*sim3*), L972 ($asf1^+$), and TYP48 (*asf1-33* mutant) harboring pREP41 or pREP41-*sim3* were diluted 10-fold from 3x 10^6 cells with sterilized water, and spotted onto EMM (with or without 2μM thiamine). Thiamine repressed the expression of *sim3* partly in pREP41, and completely in pREP81. Each plate was cultured at 26°C, 30°C, 34°C, and 36°C for 5 days.

Fig. 4. Deletion of *sim3* caused partial sensitivity to DNA replication inhibitor, HU. Logarithmically growing KT146 (h^+ *leu1-32 ura4-D18 sim3*::kanMX6) cells were diluted 10-fold from 3x 10^6 cells with sterilized water, and spotted onto YES (No drug, 18mg/ml TBZ 10mM HU, 5mM CPT, 0.0075% MMS) plates. Plates were incubated at 18, 26, 30, and 36°C for 3 days.

Table 1. *S. pombe* strains used in this study

strain	genotype	resource
L972	h^{-}	Lab stock
PR110	h^{-} *leu1-32 ura4-D18*	Lab stock
SKP605-33	h^{+} *leu1-32 ura4-D18 asf1-33-13myc-kan*^{r}	Lab stock
SKP593-33P	h^{-} *asf1-33-13myc-kan*^{r}	Lab stock
SKP561-15	h^{-} *leu1-32 ura4-D18 asf1-13myc-kan*^{r}	Lab stock
SKP593-34	h^{+} *leu1-32 ura4-D18 otr1::ura4*$^{+}$ *asf1-33-13myc-kan*^{r}	Lab stock
SKP551-1	h^{+}*leu1-32ura4-D18otr1::ura4*$^{+}$	Lab stock
SKP620	h^{-} *leu1-32 asf1-30-13myc- kan*^{r}	Lab stock
SKP593-35	h^{+} *leu1-32 ura4-D18 otr1::ura4*$^{+}$ *asf1-30-13myc-kan*^{r}	Lab stock
KT146	h^{+} *leu1-32 ura4-D18 sim3::kan*^{r}	This study
MBY1838	h^{-} *leu1-32 ura4-D18 rad3::ura4*$^{+}$	Mohan Balasubramanian
KT166	h^{-} *rad22-GFP-kan*^{r} *asf1-33-13myc-kan*^{r} *leu1-32*	Lab stock
TYP48	h^{-} *leu1-32 ura4-D18 asf1-33-13myc-hph*^{r}	this study

Table S1. Oligonucleotides used in this study

Name	Sequence
sim3 d1	TGAATGCTTAGCCATGGCTG
sim3 d2	CGTCGACCTGCAGCGTACGATTTTCGCGTAACTTTTAAAAATTAT
sim3 d3	CGAGCTCGAATTCATCGATGTTGATCACAATTAGTTGAAGTTAG
sim3 d4	TTAGGAGTAAAGGAGTTGGC
sim3 check	CTATAGGTGAACAGCGCTAC
pFA6a-F	TCGTACGCTGCAGGTCGACG
pFA6a-R	CATCGATGAATTCGAGCTCG
chk HR 42-14	GCTAGGATACAGTTCTCACA
sim3-grc1	TCGCTTTGTTAAATCATATGTCTTCTGATACGAAAACACT
sim3-grc2	TACTGGCAAGGGAGACATTCTTAATCCTTCTTTTTCTTATC
Pnmt1 80bp F	GGCATATCATCAATTGAATA
Tnmt1 80bp R	TAATATGCAGCTTGAATGGG

Materials and methods for supplementary Fig. 1.

Indirect immunofluorescence of Asf1 proteins

Indirect immunofluorescence was performed as described previously [11]. Cells were incubated in EMM (without thiamine) for 6h. After cell fixation, cells were treated with Zymolyase100T (Seikagaku) and TritonX-100. Then, blocking buffer was added to cells, and incubated for several hours. Asf1-13myc proteins were stained with anti-myc antibody (Santa Cruz Biotechnology, Inc.) and Alexafluor 488 goat anti-mouse IgG (Molecular Probes). Fluorescence of Asf1-13myc proteins was observed by a confocal laser scanning microscope (Leica TCS SP-5, Leica Microsystems, Tokyo).

Western blotting

Cellular proteins were prepared by a rapid protein extraction method from fission yeast as described previously [16]. Prepared proteins were resolved with SDS-PAGE, and transferred to PVDF membrane. Asf1-13myc proteins were detected with an anti-Myc antibody (Santa Cruz Biotechnology, Inc.). Tubulin proteins (loading control) were detected using an anti-tubulin antibody (Sigma).

Supplementary Fig.1.

Overexpression of *sim3* did not affect localization and protein level of Asf1 mutant proteins. (A) L972 (No tag), SKP561-15 (h^- *leu1-32 ura4-D18 asf1*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3*, SKP605-33 (h^+ *leu1-32 ura4-D18 asf1-33*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3,* and SKP620 (h^- *leu1-32 asf1-30*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3* were incubated in EMM medium (without thiamine) at 26 ^{o}C and 36°C for 6h. Cells were collected by centrifugation, and fixed with formaldehyde. After treatment with Zymolyase100T and TritonX-100, cells were incubated in blocking buffer. Asf1-13myc proteins were stained with anti-Myc antibody and Alexafluor 488 goat anti-mouse IgG. Observation of fluorescence was performed using a confocal laser scanning microscope (Leica, TCS-SP5). (B) L972 (No tag), SKP561-15 (h^- *leu1-32 ura4-D18 asf1*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3*,

SKP605-33 (*h*$^{+}$ *leu1-32 ura4-D18 asf1-33*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3,* and SKP620 (*h*$^{-}$ *leu1-32 asf1-30*-13myc-kanMX6) harboring pREP41 or pREP41-*sim3* were cultured in EMM (without thiamine) containing 100 μg/ml CHX at 26°C and 36°C for 6h. Cellular proteins were prepared as described in Materials and methods. Asf1-13myc proteins were detected with an anti-Myc antibody. Tubulin was used as a loading control.

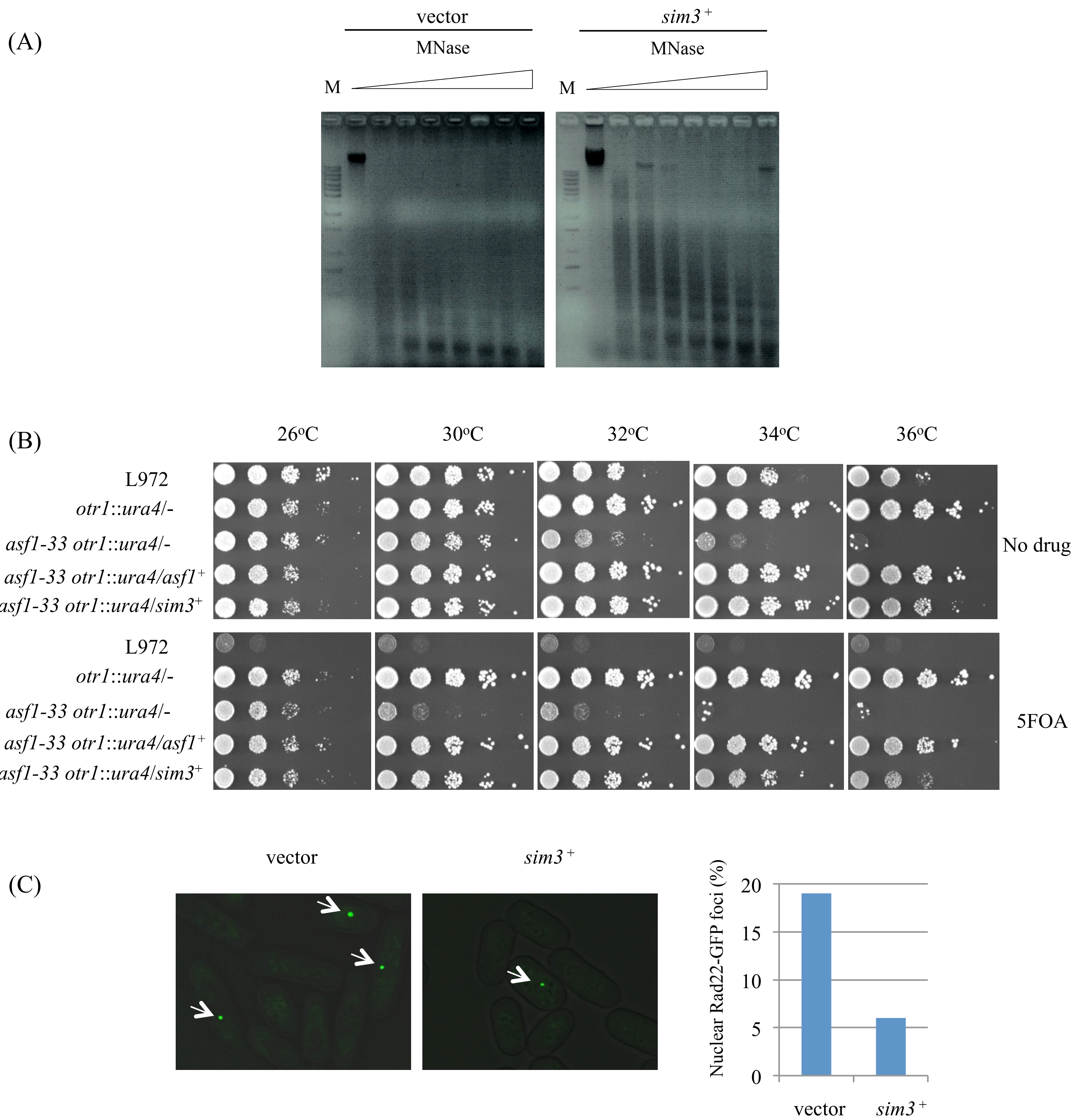


Fig.1

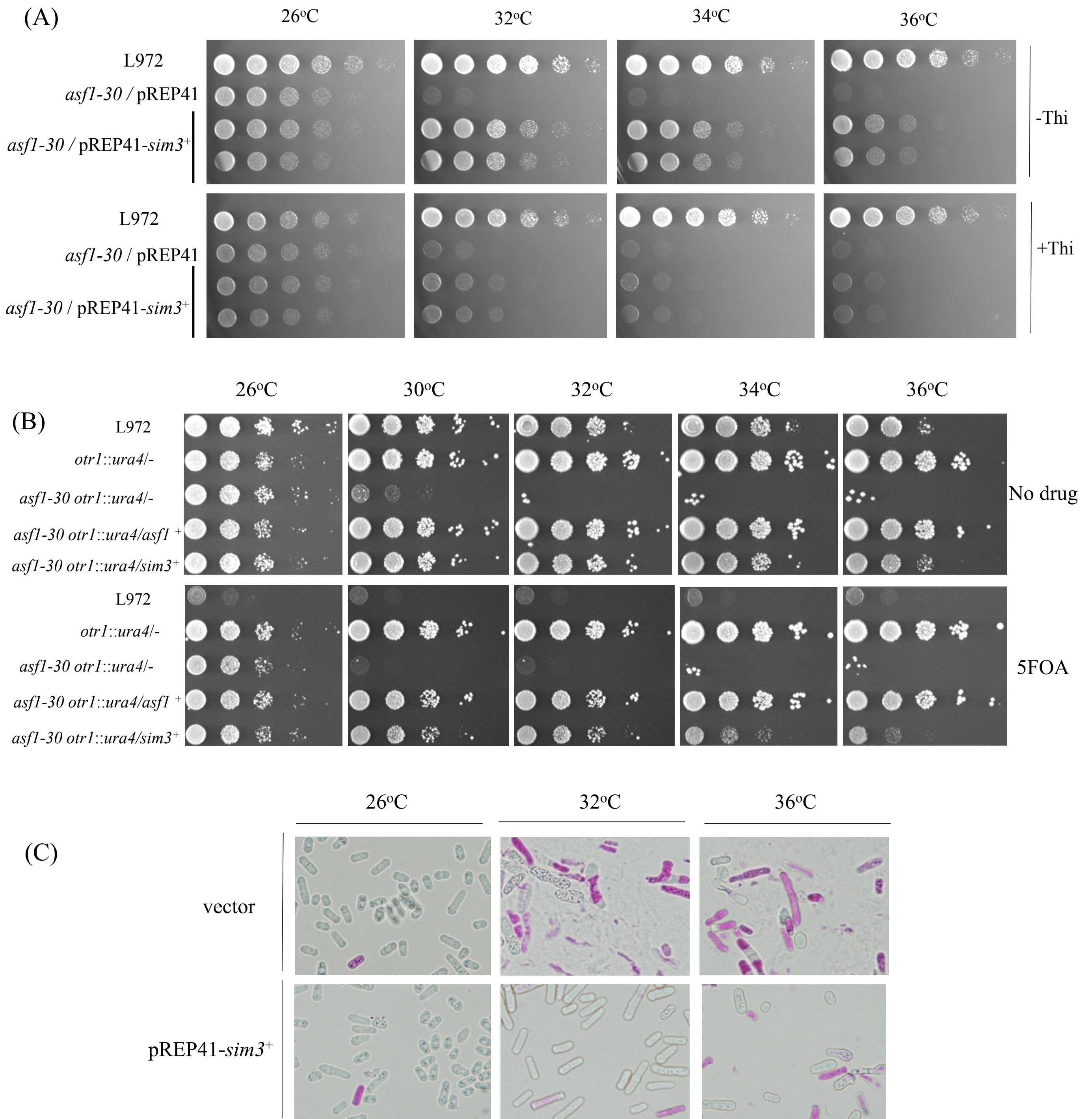
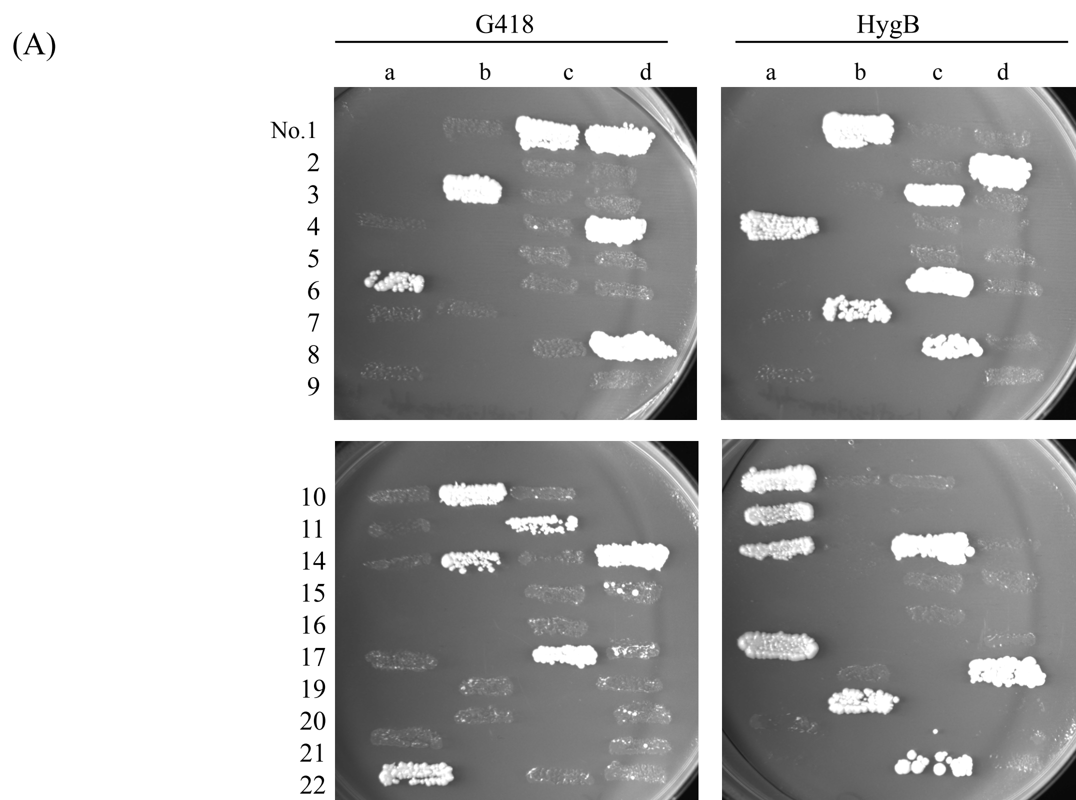


Fig.2



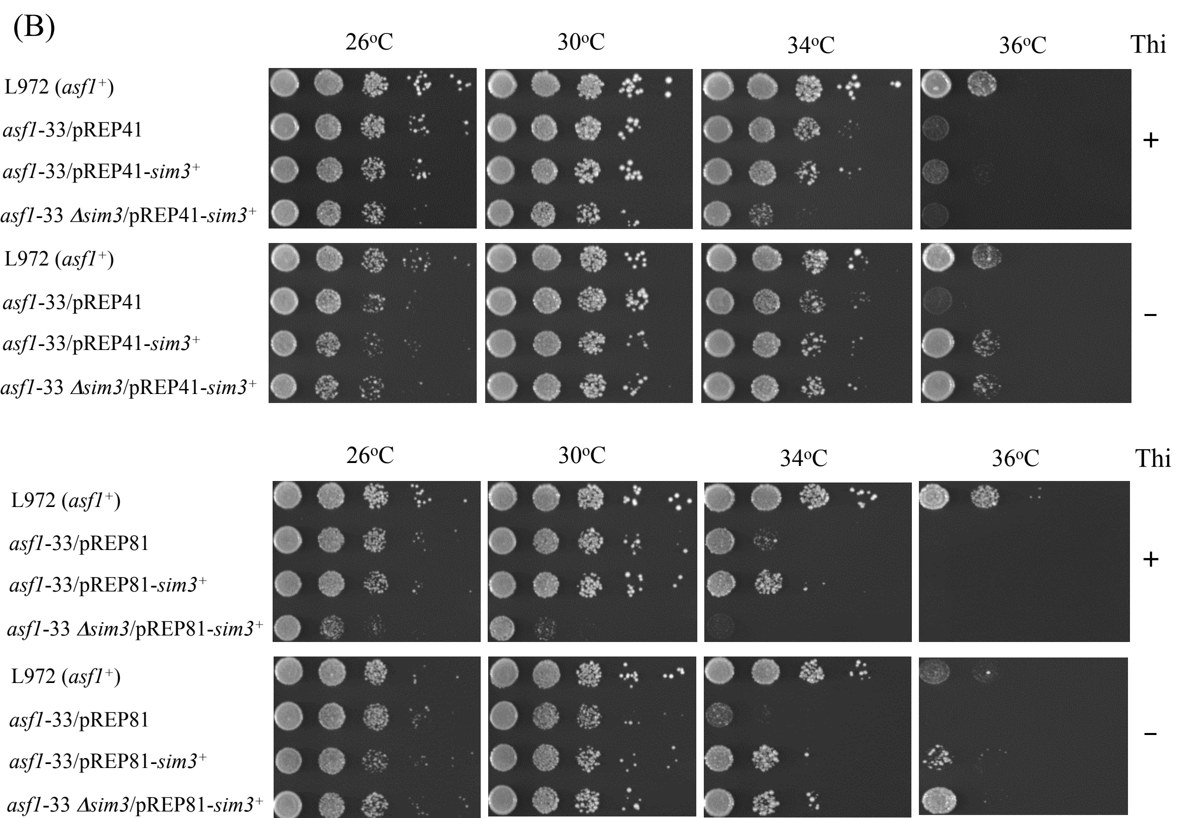


Fig.3

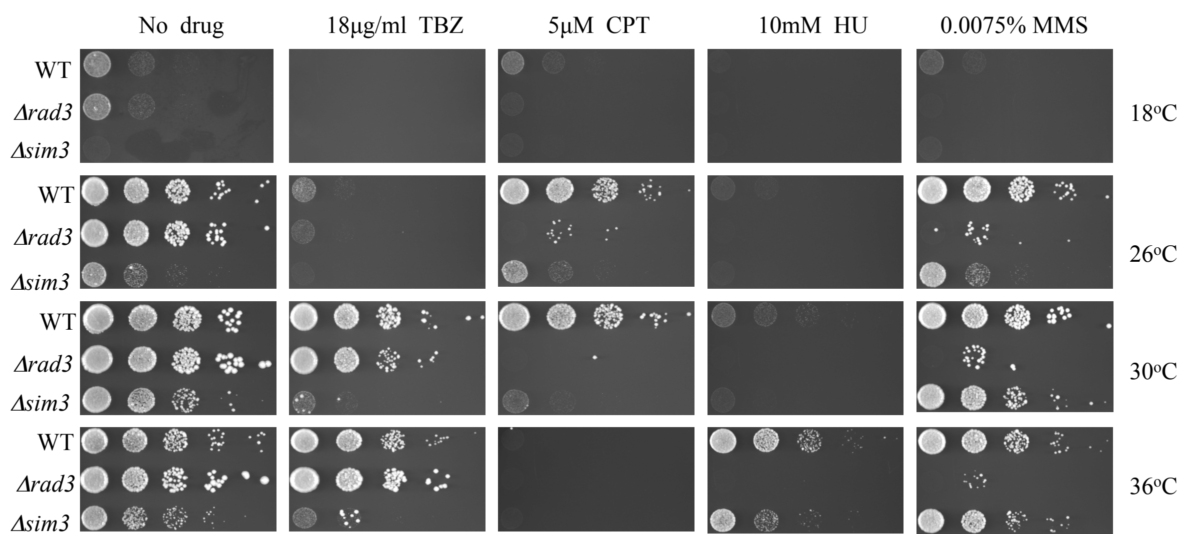
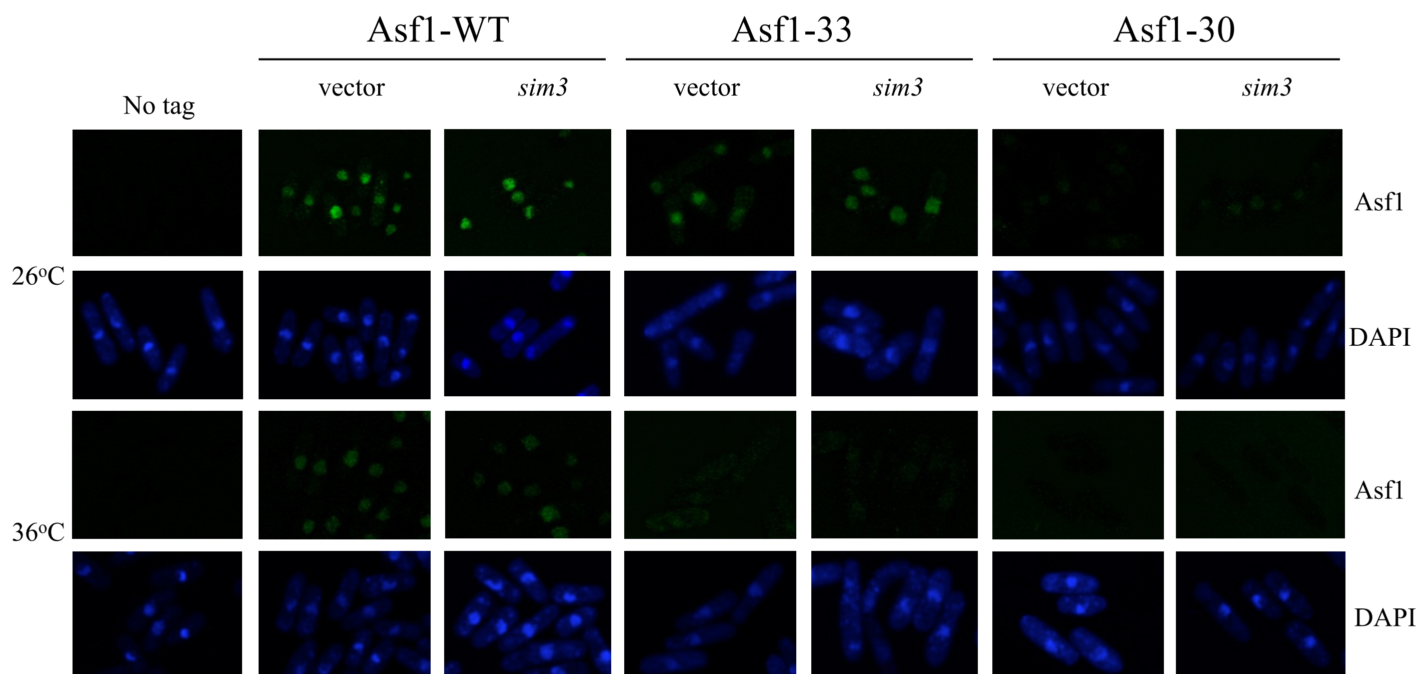
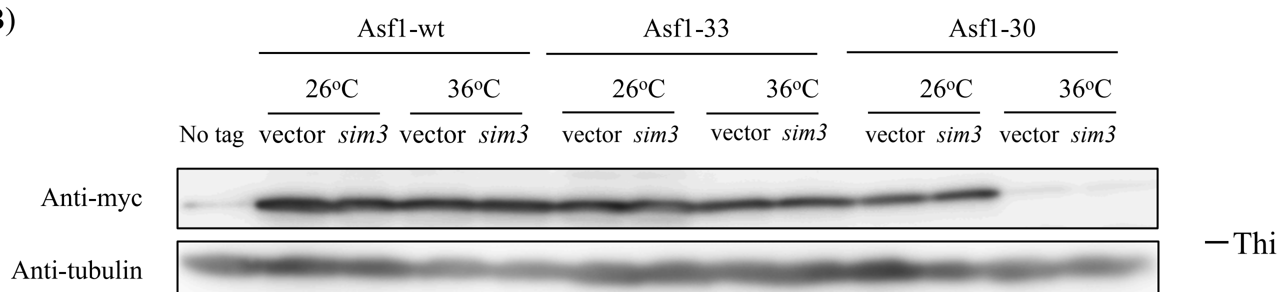


Fig.4

(A)



(B)



Supplementary Figure. 1