Title

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Functional Conservation between Fission Yeast *moc1/sds23* and Its Two Orthologs, Budding Yeast *SDS23* and *SDS24*, and Phenotypic Differences in Their Disruptants

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The *moc1*/*sds23* gene was isolated to induce sexual development of a sterile strain due to overexpression of adenylate cyclase in *Schizosaccharomyces pombe*. Here, we studied the functional conservation between *moc1*/*sds23* and its two orthologs *SDS23* and *SDS24* in *Saccharomyces cerevisiae*. We observed that the temperature sensitivity, salt tolerance, cell morphology, and sterility of the Δ*moc1* mutant in *S. pombe* were recovered by expressing either *S. cerevisiae SDS23* or *SDS24*. We found that deletion of both *SDS23* and *SDS24* resulted in the production of a large vacuole that was reversed by the expression of *S. pombe moc1*/*sds23*. In these ways we found that *S. pombe* Moc1/Sds23 and *S. cerevisiae* SDS23p or SDS24p are functional homologs. In addition we found that the Δ*sds23* Δ*sds24* diploid strain reduces cell separation in forming pseudohyphal-like growth in *S. cerevisiae*. Thus *S. pombe moc1*/*sds23* and *S. cerevisiae SDS23* or *SDS24* are interchangeable with each other, but their disruptants are phenotypically dissimilar.

Key words: *S. pombe*, *S. cerevisiae, moc1,* Vacuole, Pseudohyphae

Sexual development in *Schizosaccharomyces pombe* is primarily regulated by three signaling pathways: the cAMP pathway, the stress responsive pathway, and the pheromone signaling pathway.[1,2] When nutrient conditions are altered, *S. pombe* cells are arrested in the G_1 phase. Then the opposite mating types (h^- and h^+) mate, initiate conjugation and subsequently form zygotes to make four ascospores. Both the stress responsive pathway and the pheromone signaling pathway positively regulate the transcription of *ste11,* encoding a key regulator of sexual development.[3,4] But the cAMP pathway negatively regulates it through the action of the Rst2p transcription factor via protein kinase A.[4,5] Deletion of chromosomal adenylyl cyclase gene (*cyr1)* allows it to enter sexual development even under nutritionally rich conditions.[6] Several similar types of mutants called *sam* were isolated.[7,8] On the contrary, when the *cyr1* gene is highly expressed, *S. pombe* cells become partially sterile because the cAMP level remains high even during the stationary phase.[6] The *moc2*

gene encoding RNA helicase has been isolated as to suppress the sterility phenotype of *S. pombe* when *cyr1* is overexpressed.[9] This gene has 58% identity at the amino acid level with *Saccharomyces cerevisiae DED1,* which also encodes a RNA helicase, implicated in nuclear pre-messenger RNA splicing and also in translation.[10] In the same screening isolating *moc2*, we isolated *moc1* (also known as *sds23*[11]), which stands for multicopy suppressor of overexpressed *cyr1*.

Protein phosphatase 1 (PP1), which directly or indirectly regulates the 20S cyclosome/APC (anaphase-promoting complex), is required for anaphase–promoting proteolysis in *S. pombe*. Sds23 has been found to bypass the requirement of PP1 and cyclosome/APC for onset of the anaphase.[11] Although the *sds23* gene is not essential for cell viability, the deletion mutant cannot form colonies at a lower temperature (22°C) or a higher temperature (36°C). The progression of anaphase and cytokinesis is retarded and the cell shape is elongated in a Δ*sds23* mutant.[11] A comparison of the *moc1*/*sds23* gene and the corresponding protein in the DDBJ and Gen Bank reveals the existence of two similar genes, *YGL056c* (*SDS23*) and *YBR214w* (*SDS24*), in *S. cerevisiae,* and others in some other fungi. Both *SDS23* and *SDS24* encode a 527 amino acid long peptide with identities of 45.4% and 44.8% with the *S. pombe moc1*/*sds23* product, respectively. Those protein sequences contain a common domain, CBS (cystathionine-beta-synthase), which is predicted to have a multiple trafficking function for protein-protein interaction and metabolic regulations found in the protein like AMP activating kinase [http://smart.embl-heidelberg.de], but no other information was obtained from the sequences. *SDS23* and *SDS24* were named after it because only they are similar to *S. pombe sds23*, and no one has studied their functions extensively. Here we decided to study the functional conservation of *moc1*/*sds23* with *SDS23* and *SDS24*. We also examined the phenotype of a Δ*sds23,* Δ*sds24* mutant in *S. cerevisiae*.

The *S. cerevisiae* strains used in this study are listed in Table 1. Standard yeast culture media and genetic manipulations were used, as described previously.[12,13] *S. pombe* strains were grown in complete YEA medium (0.5% yeast extract, 2% glucose, and 0.0075% adenine) or in the synthetic

minimal medium PM (0.3% potassium hydrogen phthalate, 0.22% sodium phosphate, 0.5% ammonium chloride, 2% glucose, vitamins, minerals, and salts) with the addition of appropriate auxotrophic supplements (0.0075% adenine, leucine, and uracil) when required.[12] The *S. cerevisiae* strains were maintained on rich YPD media composed of 1% yeast extract (Y), 2% bactopeptone (P), 2% dextrose (D), and 2% agar. YPD media were used to culture all strains prior to labeling with fluorescent dye. Electroporation or the LiOAc method was used to transform yeast cells.[14,15] *Escherichia coli* DH5α grown in Luria broth medium (1% polypepton, 0.5% yeast extract, 1% sodium chloride) containing 50 μg/ml ampicillin, hosted all plasmid manipulations, and standard methods were used for DNA manipulations.[16]

First we independently constructed an *S. pombe* Δ*moc1* mutant designated TF1 (*h*^{90} *ade6-210, leu1-32 ura4-D18 moc1::LEU2),* which was derived from a diploid strain in which one *moc1* gene was replaced with *LEU2* in a diploid strain, SP826.[17] A tetrad dissection was carried out to isolate a haploid Δ*moc1* mutant. Southern blot analysis confirmed a proper disruption of *moc1* in TF1. We observed that a Δ*moc1* mutant (TF1) grew slowly at 30°C but failed to grow at 36°C, in contrast to the wild-type. The cellular shape of the disruptant was a little longer than that of the wild-type in the mitotic log phase, and the sporulation efficiency of the disruptant was very low. We also observed that the Δ*moc1* mutant was sensitive to KCl (Fig.1). All these results are consistent with those reported by Ishii *et al*.[11] but not with those reported by Jang *et al*.[18] who reported that *moc1*/*sds23* (*psp1* in their paper) is essential for growth. We then transformed the Δ*moc1* mutant with a multicopy plasmid expressing *SDS23*, *SDS24,* and *moc1* to test their recovery of temperature sensitivity and salt tolerance. We observed that overexpression of these three genes recovered the phenotype of the Δ*moc1* strain and grew as well as the wild-type at 36°C and also 0.5M KCl containing complete medium (Fig. 1A). The elongated cellular morphology and sterility were also restored by expression of either *SDS23* or *SDS24* (Fig. 1B). The result that both *SDS23* and *SDS24* can suppress the phenotypes of the Δ*moc1* mutant of *S. pombe* indicates that *SDS23* and *SDS24* have similar functions to *S. pombe moc1*/*sds23*.

We proceeded to investigate the phenotype of the *sds23* and *sds24* mutants in *S. cerevisiae*. We purchased a single mutant lacking *SDS23* or *SDS24* from Invitrogen. We checked temperature sensitivity at 30°C and 36°C and salt tolerance in 1M NaCl, but did not detect any difference between the wild-type and the single mutant of *sds23* or *sds24*. We also drew the growth curve of a mutant, but no difference in growth was found as compared with the wild-type. Because the single mutant did not display any obvious phenotypes, we constructed a double mutant of *sds23* and *sds24*. Construction of a strain with both Δ*sds23* and Δ*sds24* was accomplished by crossing ΔSDS23 (*MATa, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, sds23::kanMX4*) with ΔSDS24 (*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, sds24::kanMX4*). After tetrad dissection of spores from a diploid (Δ*sds23*/*SDS23* Δ*sds24*/*SDS24*), segregants of a NPD type (2:2), in which two spores grew on YPD+G418 medium, were selected. Genomic Southern analysis confirmed the disruption of both *SDS23* and *SDS24* genes in the genome, and those isolates were designated TN3 and TN4 (Table 1). The wild-type strains derived by the same tetrad dissection were designated TN1 and TN2 and used for comparison.

There was no difference in growth, salt sensitivity, or cellular morphology between TN1 (wild type) and TN3 (Δ*sds23* Δ*sds24*), but we found a difference in their vacuole sizes. When we cultivated cells at 30°C on YPD medium for 3 d, we found that the Δ*sds23* Δ*sds24* mutant formed larger vacuoles in about 50% of the cells, while the wild-type (TN1) formed them in about 2-3 % of the cells. Larger vacuoles were counted among 1,000 cells that possessed a significantly larger vacuole than a normal average-size vacuole. This phenotype was first observed under a light microscope and then by staining with FM4-64, a lipophilic (hydrophobic) fluorescent dye that stains vacuoles exclusively.[19,20] The stained cells were observed under the microscope using fluorescence with Rhodamine/Cy3 filter. The vacuoles inside the cells of TN3 were clearly larger than the wild-type cells (Fig. 2A). We further overexpressed *moc1* with a multicopy plasmid, pADNSΔEI, to test its suppressive effect in a diploid TN11 (Δ*sds23* Δ*sds24*). We observed that only vector-bearing cells contained roughly 60 % larger vacuoles, whereas the ones overexpressing *moc1*/*sds23* by pADNSΔEI-moc1 contained roughly 10% larger vacuoles, indicating that *moc1*/*sds23* reversed the

phenotype (Fig. 2B). We noticed that in a diploid Δ*sds23* Δ*sds24* strain some vacuoles assumed a two-layer structure (arrows), which is unusual. The larger vacuoles of the haploid strain TN3 were also reversed by expression of *moc1* (data not shown). This is our first observation that *SDS23* and *SDS24* are required for maintaining normal vacuole morphology inside the cells of *S. cerevisiae*.

In many fungi, including yeasts, nutrient deprivation or change of the environment results in a distinct morphological differentiation. Both haploid and diploid *S. cerevisiae* cells are able to switch from yeast-cell form to pseudohyphal form in response to nitrogen starvation or other stresses.[21] Multiple factors which involve the cAMP pathway are also required for this dimorphic switch.[21] Starch-degrading haploid and diploid *S. cerevisiae* cells are able to switch from yeast-cell form to a pseudohyphal form in response to nitrogen limitation and/or carbon source alterlation.[21] We used galactose instead of glucose as the carbon source to test whether any difference in cellular morphology was to be observed in Δ*sds23* Δ*sds24* cells. We cultivated cells in a YP-Galactose plate at 30°C for 3 d and observed them under the microscope. Figure 3 shows the vegetative growth pattern where the Δ*sds23* Δ*sds24* diploid strain (TN11) grew as a pseudohyphal-like growth which reached 40% of total cells observed. One thousand cells were counted, and if more than three cells were connected, we judged it to be a as pseudohyphal-like growth. The diploid wild-type (TN10) was used as a control where we did not find any branching or pseudohyphal growth except budding (Fig. 3). The haploid disruptant TN3 (Δ*sds23* Δ*sds24*) did not display any such pseudohyphal-like growth phenotype (data not shown).

In this study we found that *S. pombe moc1*/*sds23* and *S. cerevisiae SDS23*or *SDS24* are functionally interchangeable, suggesting that three proteins (Moc1/Sds23, SDS23p, and SDS24p) have biologically similar functions. We also found that the phenotypes in the disruptants of two yeasts are different. The phenotypes of the *S. pombe* Δ*moc1* mutant include low and high temperature sensitivities, salt sensitivity, reduced sporulation efficiency, and cell elongation, but those phenotypes were not observed in the *S. cerevisiae* Δ*sds23* Δ*sds24* mutant. Apparently there is a discrepancy in the phenotypes of Δ*moc1*/*sds23* mutants between the result of Ishii *et al*.[11] and Jang *et*

al.[18] But our observations support the former results. The phenotypes of the Δ*sds23*Δ*sds24* mutant include vacuole enlargement and pseudohyphal-like growth in diploid cells, but those were not found in the *S. pombe* Δ*moc1* mutant. There was one common property in that the diploid Δ*sds23* Δ*sds24* mutant was lower in sporulation efficiency (data not shown), but this might be due to a secondary effect of larger vacuole formation. These differences in the phenotypes of the disruptants of the two yeasts are not easily explained, but, there are some hints as to the functions of these proteins. One of the possibilities is that these proteins might be involved in the pathway antagonizing the cAMP pathway, because *moc1*/*sds23* was originally isolated as a suppressor of a strain in which *cyr1* is highly expressed [9] or the suppressor of the *dis2*$^-$ mutant.[11] Consistent with the idea that Moc1/Sds23 might down-regulate the cAMP pathway, it is known a *dis2*$^-$ mutant is suppressed by expression of cAMP phosphodiesterase.[22] There is also a well-known link between the cAMP pathway and pseudophyphal growth in *S. cerevisiae*. Activation of the RAS-cAMP pathway induces pseudophyphal growth which might be up-regulated by deletion of *SDS23* and *SDS24* in *S. cerevisiae*. In addition, the finding that PPI (a *dis2* ortholog) is required for vacuole fusion in *S. cerevisiae* [20] might be relevant to the observation of vacuole enlargement in a Δ*sds23* Δ*sds24* mutant. These observations reflect some common properties of Moc1/Sds23, SDS23p, and SDS24p, but further study is required to identify the functional roles of those proteins.

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Fig. 1. *S. cerevisiae SDS23* and *SDS24* are Functional Homologs of *S. pombe moc1/sds23*.

A, Suppression of slow growth, temperature sensitivity, and salt sensitivity of the Δ*moc1* mutant by *moc1, SDS23,* and *SDS24*. Transformants were diluted and spotted onto a PMA plate at the indicated temperatures and salt concentrations. B, Suppression of morphology and sterile phenotype in the Δ*moc1* mutant by *moc1, SDS23,* and *SDS24*. The *moc1* gene was first amplified by PCR from pMCS24, a derivative of pWH5.[9] Amplified *moc1* DNA was digested with *Not*I-*Bam*HI and ligated at the same site of pBluescriptII SK- vector. To construct pSLF173-moc1, pBluescript-moc1 was digested with *Not*I-*Sma*I and then ligated with at same cloning site of pSLF173 [23,24] vector. In a similar way, the genes for *SDS23* and *SDS24* were amplified by PCR from

S. cerevisiae chromosome and ligated with pSLF173 at the *Not*I-*Bgl* II site to yield pSLF173-SDS23 and pSLF173-SDS24.

Fig. 2. Double Disruption Causes a Larger Vacuole in *S. cerevisiae*.

A, TN1 (wild-type) and TN3 (Δ*sds23* Δ*sds24*) cells were stained with FM4-64,*N*-(3-triethylammoniumpropyl)-4-(6-(4-diethylamino) phenyl) hexatrienyl) pyridinium dibromide, a lipophilic styryl dye. Cells were grown logarithmically in liquid YPD overnight (OD_{600} < 2) at 30°C. The cells were harvested (2 min, 3,000*g*), re-suspended in YPD with 10 μm FM4-64 at OD_{600} = 1, and shaken for 3 h at 30°C. The cells were harvested as above, resuspended in YPD at OD_{600} = 0.2, and shaken for 2-3 h at 30°C. Individual samples were removed from the shaker and harvested (1 min, 3,000*g*), resuspended in YPD at OD_{600} = 10, and immediately observed with a fluorescence microscope. B, *S. cerevisiae* expression vector, pADNSΔEI (one *Eco*RI restriction site was deleted from pADNS [25] of its *LEU2* region) carrying 2 μ plasmid origin of replication for propagation, the *ADH* promoter sequences for the expression of introduced genes, and a *LEU2* marker. The *Hind*III-*Eco*RI fragment of *moc1* was amplified from pMCS24 plasmid by PCR reaction. An amplified fragment and pADNSΔEI were digested with *Hind*III and *Eco*RI to ligate, yielding the construct pADNSΔEI-moc1. TN11, a Δ*sds23* Δ*sds24* diploid was transformed with pADNSΔEI-moc1 in an electroporation procedure. The transformants were then cultured onto SC-L medium for 3 d at 30°C. The cells were then stained as previously described with FM4-64 for microscopic observation. Arrows indicate a two layer structure.

Fig. 3. *SDS23* and *SDS24* Are Involved in Cell Separation during Budding of *S. cerevisiae*.

Wild-type (TN10) and TN11 (Δ*sds23* Δ*sds24*) cells were maintained on rich YPD media. A single colony was picked up and streaked onto a YP-Galactose (1% yeast extract, 2% bactopeptone, 2% galactose, and 2% agar) plate at 30°C for 3 d. A single colony was picked up and dissolved in sterile water. Cells were observed under a light microscope and photographs were taken. Three

individual colonies were checked for pseudohyphal growth measurement. Bar, 10μm.

Table 1. *Sachharomyces cerevisiae* Strains Used in This Experiment

Name	Genotype	Source
TN1	*MATa, his3Δ1, leu2Δ 0, ura3Δ 0, met15Δ 0, lys2Δ 0*	This study
TN2	*MATα, his3Δ1, leu2Δ0, ura3Δ0*	This study
TN3	*MATa, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, sds23::kanMX4 sds24::kanMX4*	This study
TN4	*MATα, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, sds23::kanMX4 sds24::kanMX4*	This study
TN10	*MATa/α, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0, met15Δ0/MET15, lys2Δ0/LYS2,*	This study
TN11	*MATa/α, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0, met15Δ0/MET15, lys2Δ0/LYS2, sds23::kanMX4/sds23::kanMX4, sds24::kanMX4/sds24::kanMX4*	This study
Δ*SDS23*	*MATa, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, sds23::kanMX4*	Invitrogen
Δ*SDS24*	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, sds24::kanMX4*	Invitrogen

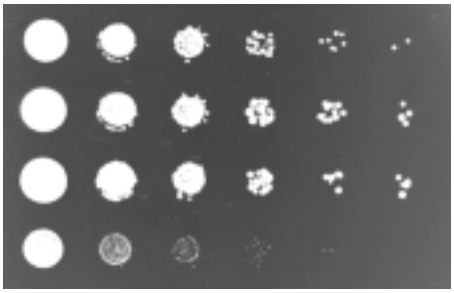
A

Δ *moc1*/pSLF173-*moc1*

Δ *moc1*/pSLF173-*SDS23*

Δ *moc1*/pSLF173-*SDS24*

Δ *moc1*/pSLF173



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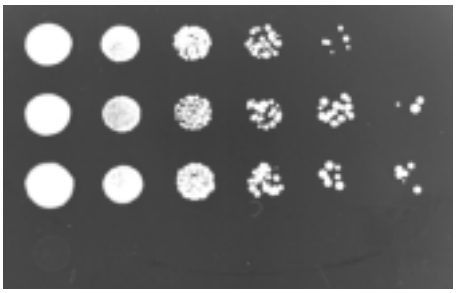

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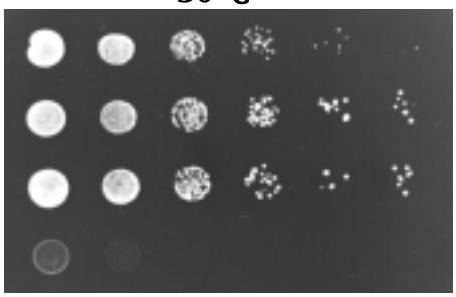

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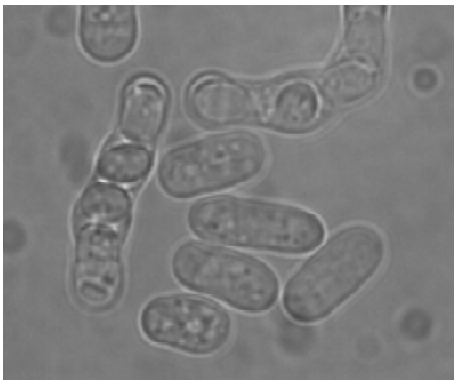
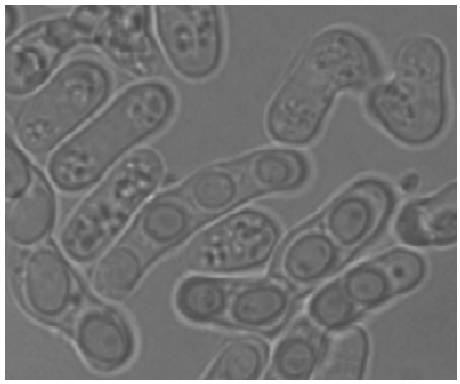
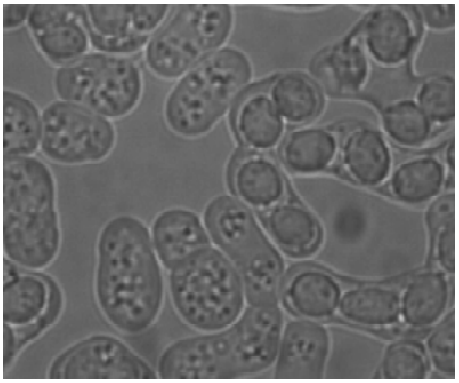
B



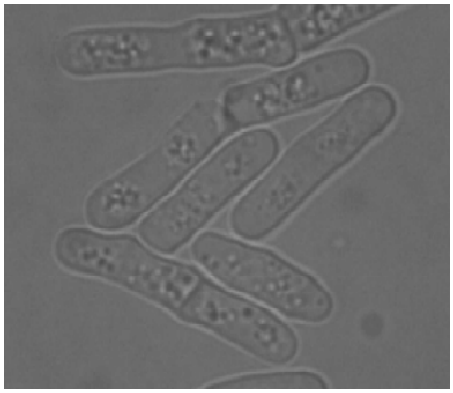
Δ *moc1*/pSLF173-*moc1*



Δ *moc1*/pSLF173-*SDS23*



Δ *moc1*/pSLF173-*SDS24*



Δ *moc1*/pSLF173

Fig. 1

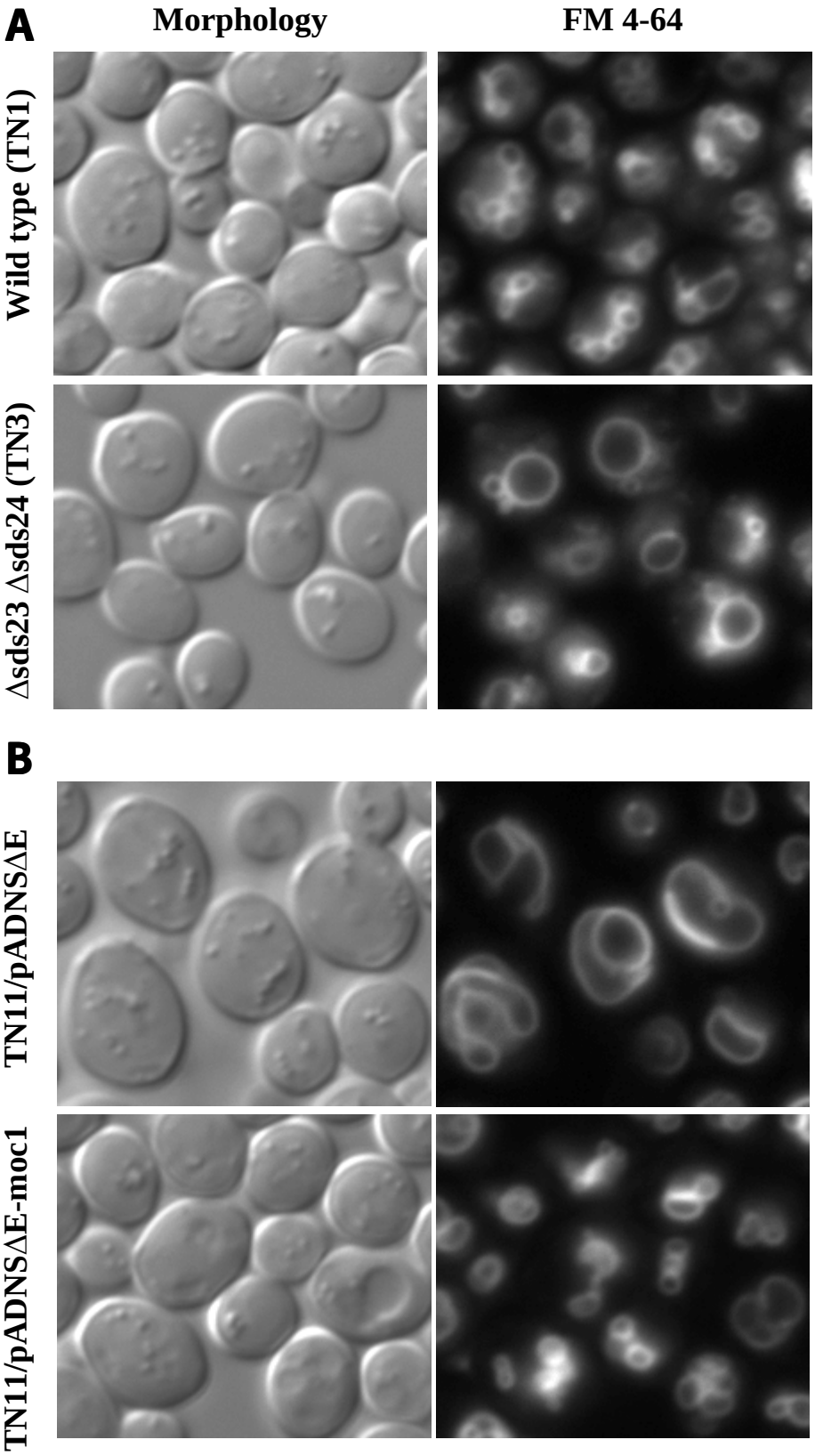
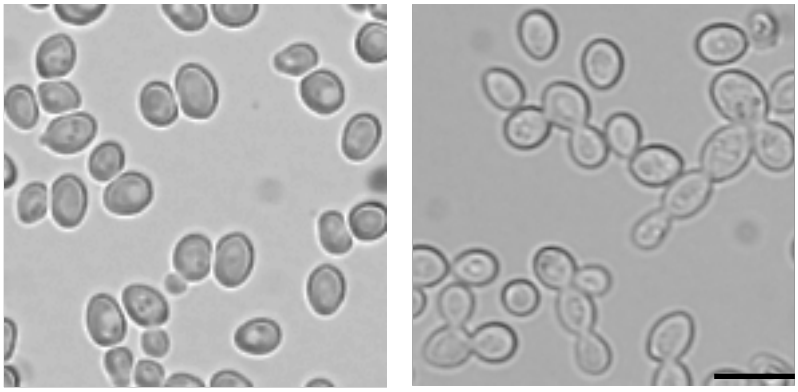

A
Morphology
FM 4-64
Wild type (TN1)
Δsds23 Δsds24 (TN3)
B
TN11/pADNSΔE
TN11/pADNSΔE-moc1

Fig. 2



Wild type (TN10) **Δ*sds23* Δ*sds24* (TN11)**

Fig.3