

**Supplemental Table 1.** Oligonucleotides used in this study

Designation	Oligonucleotide
oKK60	<u>CGTATACTTTACATTGATCACGTTTGC</u> ACTATAGACTGAATTTAAATT <u>AGAATTTTAT</u> CGGATCCCCGGGTTAATTAA ( <i>ZDS2</i> flanking sequence underlined)
oKK62	<u>GTGGCCTTATATAGGTATCTATCAATCTTG</u> TAAACAGTTATGAGTTAA <u>ATGTCTGGGAATTCGAGCTCGTTTAAAC</u> ( <i>ZDS2</i> flanking sequence underlined)
oKK135	CGTGTCGTCAAGAGTGGTC
oKK148	CAGTGGATCCAGCAGCTGTATAAGGAGTG (BamHI site underlined)
oKK149	TAGT <u>GAGCTCG</u> CTGTATTGGACAGATCATT (SacI site underlined)
oKK205	GAGTGATATCTATACTGTCG
oKK210	ATCTCAACTTTGCCTCCAAG
oKK229	GTACGAAGCTTATAATTATAATGCGGAAAAAATG (HindIII site underlined)
oKK230	AGTGCAGGAAGGATTT <u>CAGCACAAAACA</u> ATTTTGATTTTAAATTC (XmnI site underlined)
oKK247	<u>TTCTACTTATTAAGATCAATAGGCACGTGCTATTTTC</u> GAACATCCACTT <u>TCAATATGGACATGGAGGCC</u> CAGAATACCC ( <i>RTS1</i> flanking sequence underlined)
oKK248	<u>TGGCATGCCCTAAACTTCTCACTTCTTCGAGCTTG</u> TAATGAATTGCTG <u>TTTCACTGCAGTATAGCGACCAGCATT</u> CAC ( <i>RTS1</i> flanking sequence underlined)
oKY6	GCAAAGTTGAGACTTAAGGGGCAGTAGG
oKY7c	CCTACTGCCCCCTAAGTCTCAACTTTG
oKY8	GATTTGAGGGGCAGTAGGTC <u>GCGA</u> AGTGTTGTCTTCTCTC (NruI site underlined)
oKY9	GAGAGAAGACAACACTT <u>CGCGAC</u> CTACTGCCCTCAAATC (NruI site underlined)
oKY12	ACCACCATGGTACTGATGGAAGATATG (NcoI site underlined)
oKY13	TCTCTGCAGGTTCGAGGCCCTTATATAGGTATCTATC (PstI site underlined)
oKY20	GAATTTTATGGTACTGATGGAAGATATGCAAACAAAGATGGCTGC <u>AGGTCGACAACCCTTAAT</u> (Underline indicates complement to 9x-myc cassette of pOM22)
oKY21	GAGTGATATCTATACTGTCGTCGACATTAGTAGCATCGCCCTCTTCGC <u>GGCCGCATAGGCCACT</u> (Underline indicates complement to 9x-myc cassette of pOM22)
oKY31	GGCTGCAAAATGGTCACATTG
oKY32	CTTGTATTTCTCCTTCTTGTTG
oKY33b	<u>C ATATCGAGGTCAA</u> ACTGGAGAGATCTTACGCATAAGAAATA <u>TAATATAGCGCACACGGATCCCCGGGTTAATTAA</u> ( <i>CDC55</i> flanking sequence underlined)
oKY34b	<u>GTATGTGGGGAAGATATGGGATAAAAAA</u> AGTAAGGGAAAAT <u>AAGGAATTATTATAATGAATTCGAGCTCGTTTAAAC</u> ( <i>CDC55</i> flanking sequence underlined)

oKY35 GGGAAAAC TAGTGTTTCTATA  
oKY49 CTGCAGCGAGGAGCCGTAAT  
oKY50 GAAGGGATCCATGGTACTGATGGAAGATATG (BamHI site underlined)  
oKY54 GAAGGGATCCATGTCCAATAGAGATAACG (BamHI site underlined)  
oKY55 GAAGACTAGTGTATATGTCTATGTATGCAGC (SpeI site underlined)  
oKY56 GAAGGGATCCCATATGCATAGCAACGTATTTG (BamHI site underlined)  
oKY76 GAAGAAGAAGAGCTCGGTTTCGATACTCTTTATTCGA (Sac I site  
underlined)  
oKY77 CGGATATATGCGGTGTGAAAT  
oKY83 ATGGGTCGCGGA TCCGTCATTGAACCAGAAGTGAGA (BamHI site  
underlined)  
oKY87 CGCAAGCTTGTCGACGAATTCGGCCTTATATAGGTATCTATC (Sall site  
underlined)  
oKY97 GGAGTTTGCACAAGACAAGACTATT  
oKY99 GTTTTGGTGAAGTGATGAAAGAAGT  
oKY100 ACCTCATAAAATCTAGCCAACATATCGAGGTCAAACCTGGAGA  
GATCTTACGGATCCCCGGGTTAATTAA (*CDC55* sequence underlined)  
oKY101 TTGAATTCAAGTTCAATTTAAATTTCAATTAACACAGTAGTAGT  
ATGTGGAATTCGAGCTCGTTTAAAC (*CDC55* sequence underlined)  
oKY103 CTCAGGCCACAAACAACCATGTCCA  
oKY106 CTTACCTGTCAATACCGTC  
oMR1 CACCTGCTCCATGTATAAG

# Supplemental Figure 1

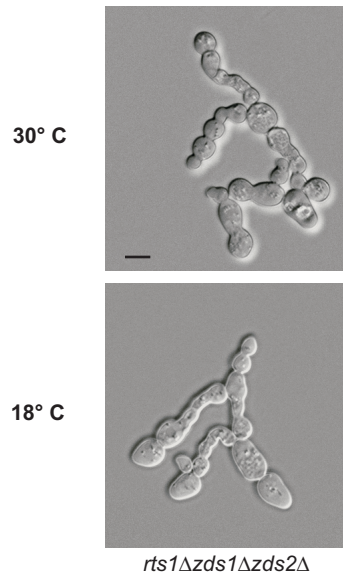
```
Sc.Zds2 (808-913) SPTAPQIS-----TLPPRKLTFEDVVKP-----DYSNAF IKFTD S AFGFPLF MITNSTVIMFDHRLG INVERAIYRLSHLKLSD DGEEL RQQVLLSNFMYSYLNLVNHT-----LYMEQVG
Sc.Zds1 (795-902) FAAPPLKH-----TSILPPRKLTFADVKKP-----DKPNS EVQFTD S AFGFPLF LLTVSTVIMFDHRLP INVERAIYRLSHLKLNSK KGLRE QVLLSNFMYAYLNLVNHT-----LYMEQVA
S.pombe (753-842) SSSPPHNDAPPKAKPISAFSELPNITSVAAEAKCQ-----TVTDD EGT DQQ S DEKSTEFKTFIPDKDYYSFPICTERAIYRLSHLKLNSA HEP LFQQVLLSNFMYSYLDLISRIS-----SNRPMNNVQ
A.gossypii (877-992) HPSLDPGKSA L GFG EIAHSLPPRKLRFDDVLEP-----EKPN S FPKFT S AFGFPLF PLTVSTVIMFDHRLP IYVERAIYRLSHLKLSD KKEEL RQQVLLSNFMYSYLNLVNH S-----LYLQQIE
C.glabrata (719-817) DSSEKTSK-----KPLYPPRKLTFADVKKK-----DKANAF IEF TDS AFGFPLF ELTYSTVIMFDHRLP IHVERAIYRLSHLKLSDPKKVALRE QVLLSNFMYSYLNLVNHT-----LYMEQKS
K.lactis (815-920) QGTLEN-----QYFLPPRKLTFADVLEP-----DRPNS EMKFTD S AFGFPLF PLTISTVIMFDHRLP IHVERAIYRLSHLKLSDPKKVALRE QVLLSNFMYAYLNLVNH S-----LYLQQIE
Y.hypolitica (1234-1359) ESSDGYMDPAQQQPMYSFIDEALFDLPSTYMKFN-MMTVM DYETPIFYSGPAVLVFP GAGRAMVMYHFFELHIERAIYRLSHLKLANP RFP LFQQVLLSNFMYAYLDL INQYQQQQQMEAGAG
C.albicans (1454-1574) LATEQSVKFSQGEDLLSKNEDKEKLDIQEKLKKSIKRTSRANQIEEF TDS AFGFPLF PPSQSLVMLDYKFEVHVERAIYRLSHLKLANPKKSLRE QVLLSNFMYAYLNLVDHT-----LHLEQQN
```

## Supplemental Figure 2

\*

<i>P.tricornutum</i> (485-527)	F P L Q L K Q R T P I R V L H R R S N M I R V R H V L S A Q A H R I D . . . . . D H Y F R L . H I
<i>Z.mays</i> (35-79)	K E M E I V Q K T P I R V L H R R S P L E R K R I I H W M E I E K I T G . . . . . S S N Y Y L L . H L
<i>E.siliculosus</i> (498-540)	E E L V V Q Q A T P I R V L H R R T L L D R P R S I F G M R A E W I N . . . . . E H F F Q L E L A
<i>S.niekkendorffii</i> (408-452)	K E L E I Q Q K T P V R V L H R R S P L V R P R I I H W M R C E A L H D . . . . . S S C Y F L L . H L
<i>H.sapiens</i> (436-478)	K D L K I D Q K T P L R V L H R R P L A V R A R V I H F M E T Q Y V D . . . . . E H H F R L . H L
<i>A.thaliana</i> (415-458)	K E L K I L Q K T P V R V L H R R S P L D R E K I I H W M K V E K I K G . . . . . H S H Y F L L . H L
<i>A.mellifera</i> (338-394)	Y V L K I I Q K T P I R V L H R R S L I N R E R K I F Q I K L I Y L H . . . . . K H F S L L . Y L
<i>P.falciparum</i> (485-527)	K H L K I I Q K T P M R V L H R R P L S P R E R L I Y E M R A R W V K P Q E L K N L N I S S E D A N I F F I L . D I
<i>H.magnifapapillata</i> (167-209)	K N I V L M Q K T P I R V L H R R P L I T R E R T I Y E T N L T R V D . . . . . E F H Y D L . F I
<i>C.elegans</i> (365-407)	I P L K I V Q K T P V R V M K R R A L L D R E R W I Y S M N F L K L D . . . . . D H H F E V . R L
<i>E.histolitica</i> (368-410)	K D L L I Q Q K T P I R V L H R R S L L T R E R M I Y S L E L H P I D . . . . . G H F G L L . E L
<i>Sc Zds1</i> (836-878)	F P L P L L T V S T V I M F D H R L P I N V E R A I Y R L S H L K L S . . . . . N S K R G L . R E
<i>Sc Zds2</i> (853-895)	F P L P M I T N S T V I M F D H R L G I N V E R A I Y R L S H L K L S . . . . . D P G R E L . R Q
<i>S.pombe</i> (815-844)	. . . . . Y W S R F P I C T E R A I Y R L S H I K L S . . . . . N A H R P L . F Q
<i>C.neoformans</i> (578-620)	A P P S P S P T S P N F Q N Y A R Y P I H V E R A V Y R L S H I K L A . . . . . N A R R P L . Y E

## Supplemental Figure 3



**Supplemental Figure 1.** Alignment of amino acid sequences with high homology to the ZH4 domain of Zds2p (residues 813-912) from representative fungal species (Accession numbers are as follow. *S. cerevisiae* Zds1 NP\_014000.1, *S. cerevisiae* Zds2 NP\_013598.1, *S. pombe* NP\_594632.1, *A. gossypii* NP\_982357.1, *C. glabrata* XP\_447996.1, *K. lactis* XP\_453144.1, *Y. lipolytica* XP505344.1, *C. albicans* XP\_717304.1). BLAST (Altschul *et al.*, 1990) was used to identify proteins that are homologous to Zds2p (813-912) and Tcoffee (Notredame *et al.*, 2000) alignment program was used to align the ZH4 domain. Asterisks mark the substituted amino acids (R863 and V868) in temperature-sensitive Zds2-3p. Dark blue shading indicates complete conservation of the residue whereas light blue shading indicates a semi-conserved residue.

**Supplemental Figure 2.** Alignment of amino acid sequences with some identity to a portion of the ZH4 domain of Zds2p (residues 853-895) from representative species (Accession numbers are as follow. *P. tricornutum* XP\_002179385.1, *Z. mays* NP\_001131786.1, *E. siliculosus* CBN75013.1, *S. moellendorffii* EFJ27746.1, *H. sapiens* NP\_653310.2, *A. thaliana* NP\_173466.2, *P. falciparum* XP\_001348196.1, *A. mellifera* XP\_001348196.1, *H. magnipapillata* XP\_002155895.1, *C. elegans* NP\_496819.1, *E. histolytica* XP\_655941.1, *S. cerevisiae* Zds1 NP\_014000.1, *S. cerevisiae* Zds2 NP\_013598.1, *S. pombe* NP\_594632.1, *C. neoformans* XP\_571315.1. BLAST (Altschul *et al.*, 1990) was used to identify proteins with sequence similarity and Tcoffee (Notredame *et al.*, 2000) alignment program was used to align the residues of Zds2p (853-895) found to have some identity across multiple Kingdoms. The residue marked with an asterisk denotes R863 in Zds2p and a conserved R in Pus10 family proteins in animals that is modeled to be involved in RNA binding (McCleverty *et al.*, 2007). Dark blue shading indicates a high degree of conservation whereas light blue shading indicates a semi-conserved residue.

**Supplemental Figure 3.** *zds1Δ zds2Δ* bud morphology defect does not require *RTS1*. Deletion of *RTS1* in a *zds1Δ zds2Δ* strain did not suppress the mutant, elongated bud phenotype at either 30°C or 18°C. DIC micrographs of representative log-phase *rts1Δ zds1Δ zds2Δ* (KKY1218) cells cultured at 18 or 30°C in rich medium (YPD) for 4h, after initial culturing at 30°C. Scale bar represents 5 microns.