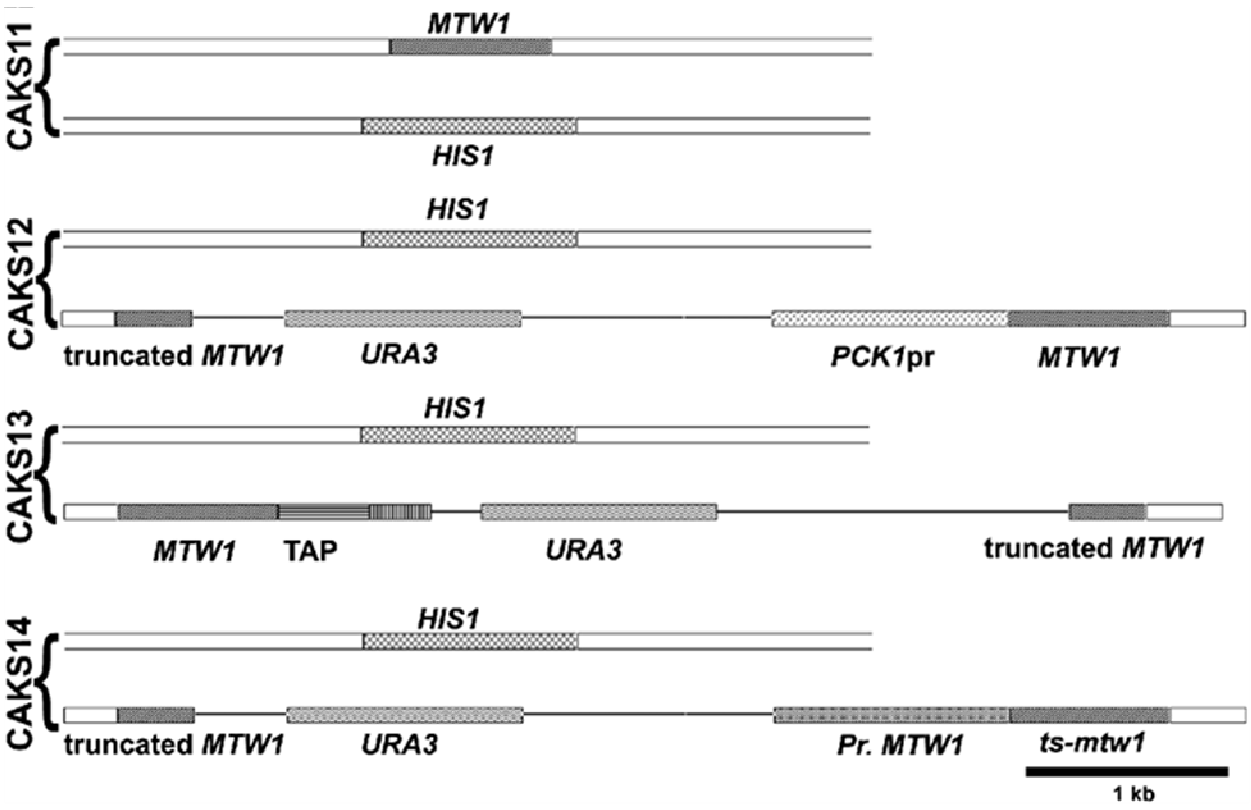
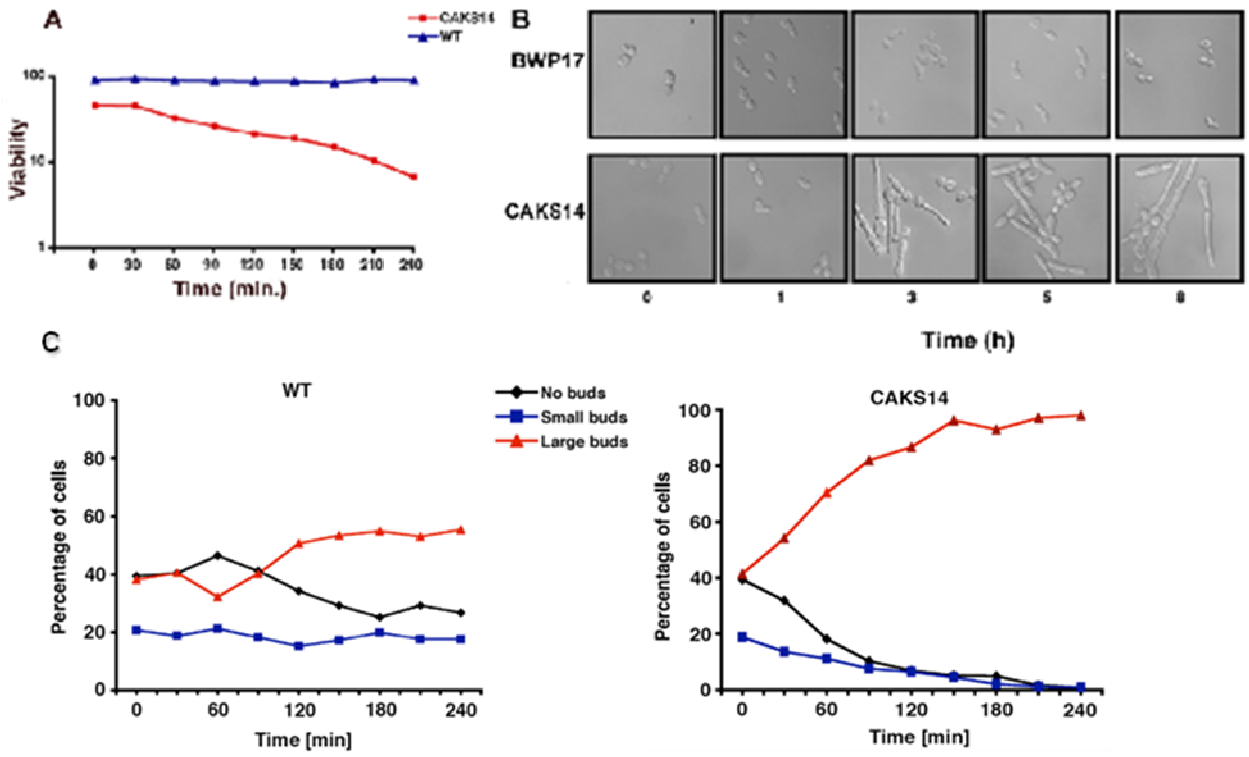


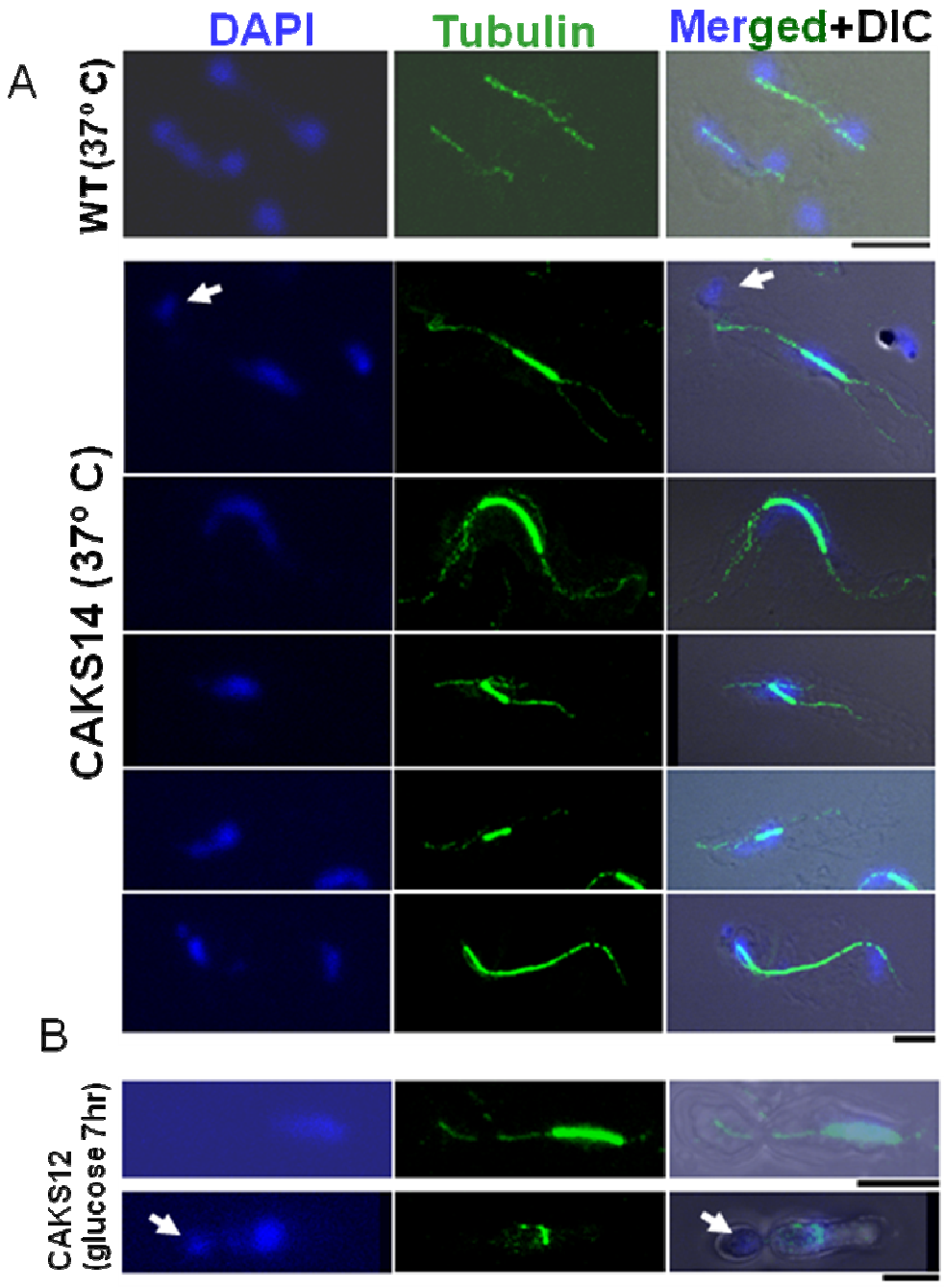
Supporting Figure S1



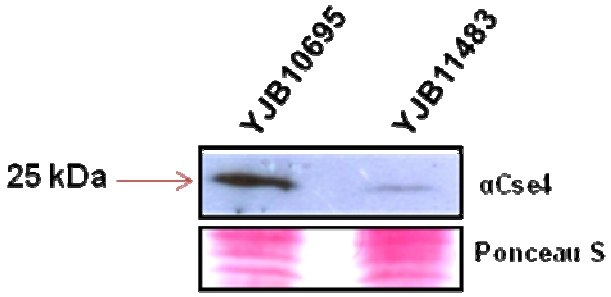
D

Strain	Undivided	Early Bud	No segregation	Arrested	Complete segregation	Total Counted
BWP17	25.5	32.5	12.4	3.8	25.5	129
CAKS14	10	12.5	47	13	16.2	191

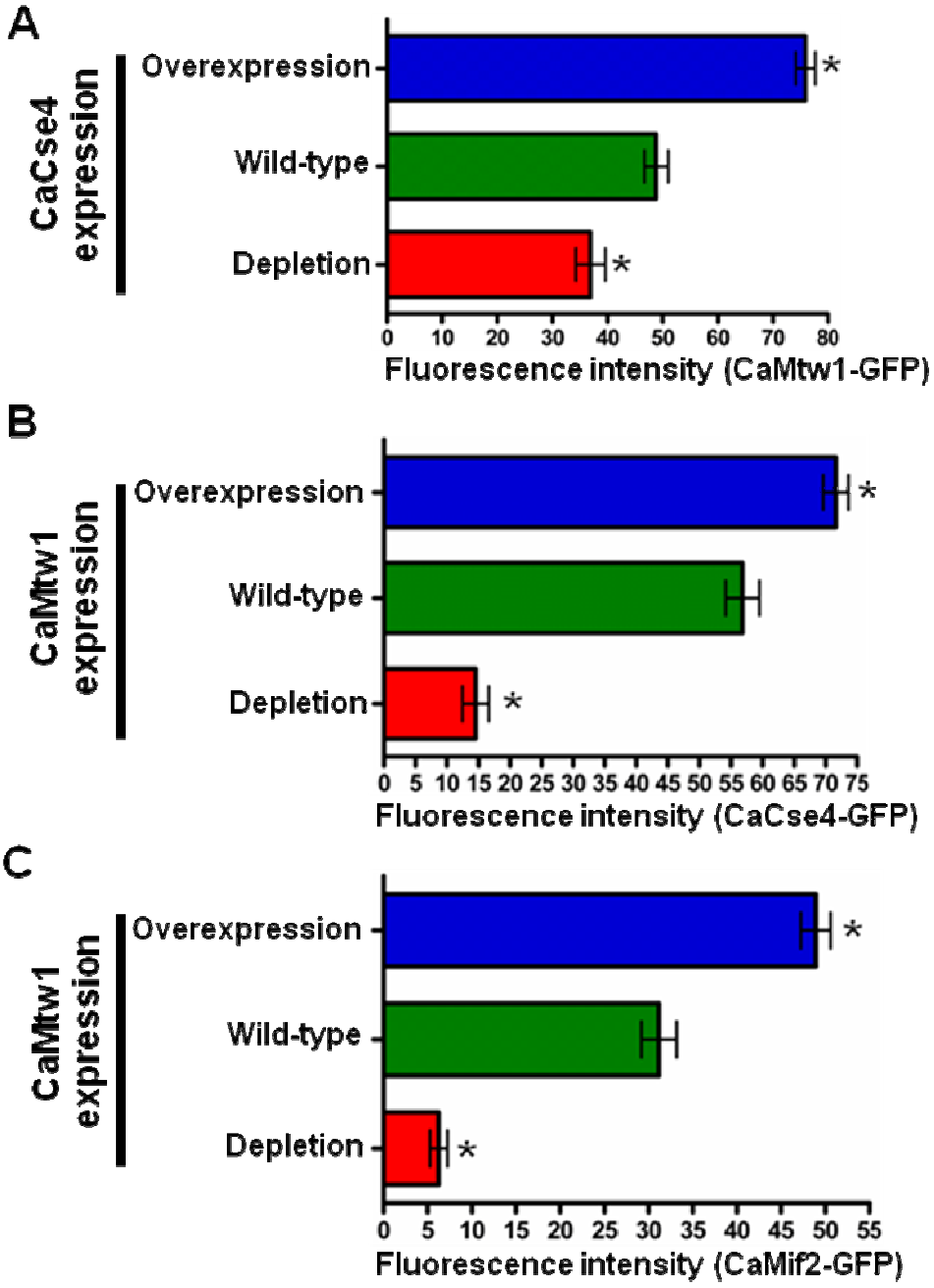
Supporting Figure S2



Supporting Figure S3

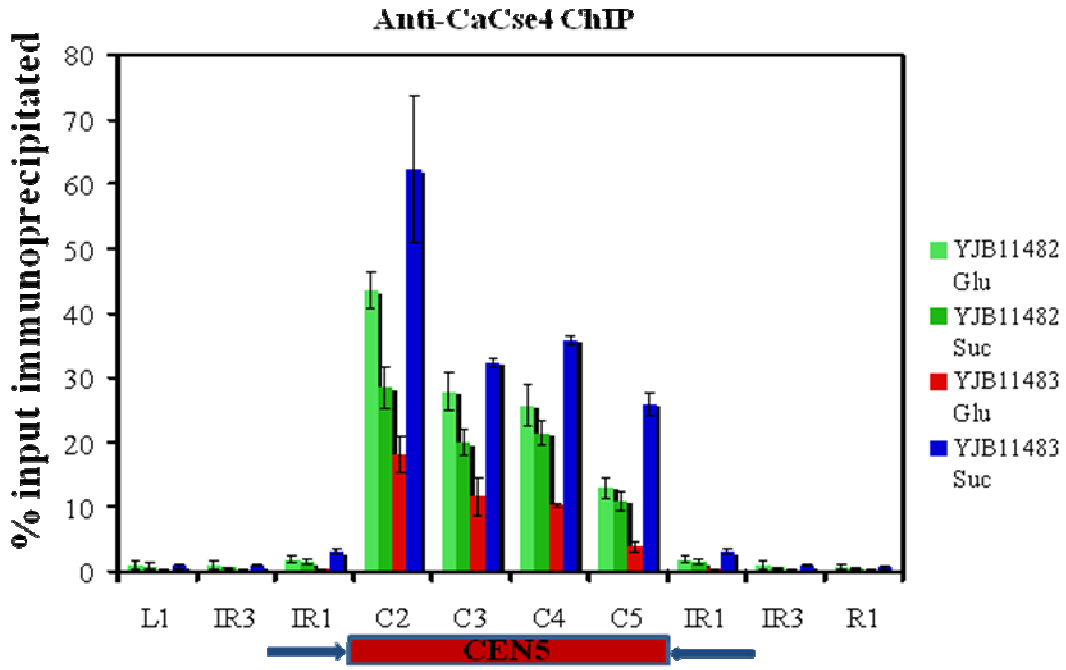


Supporting Figure S4

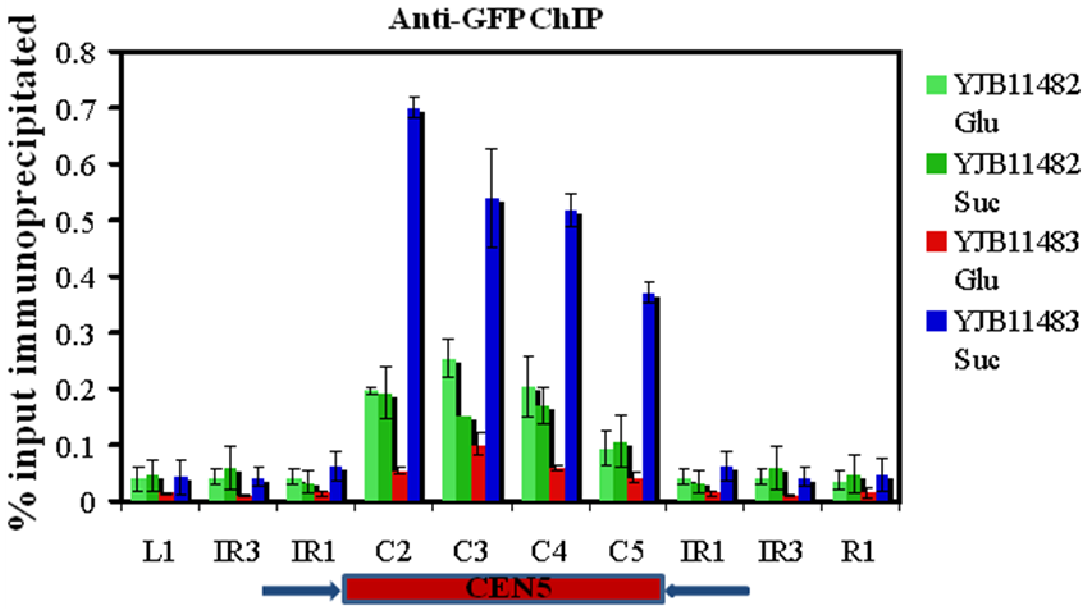


Supportng Figure S5

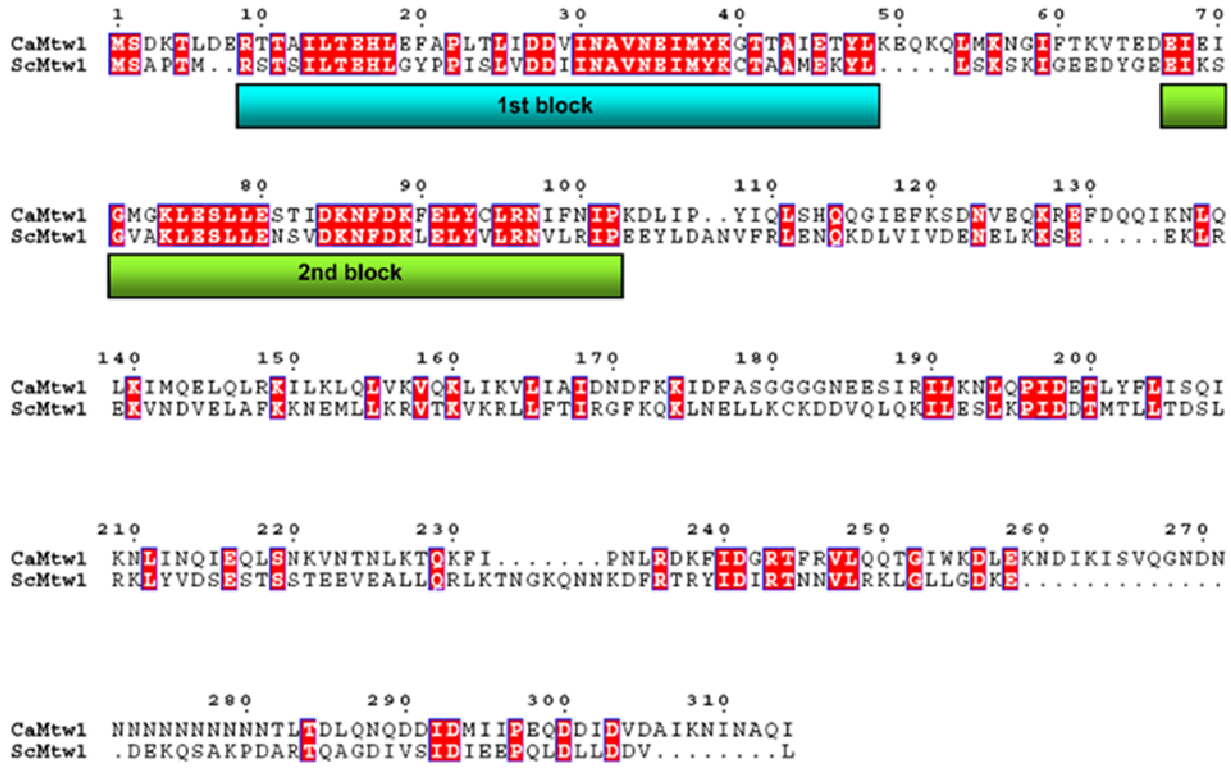
A



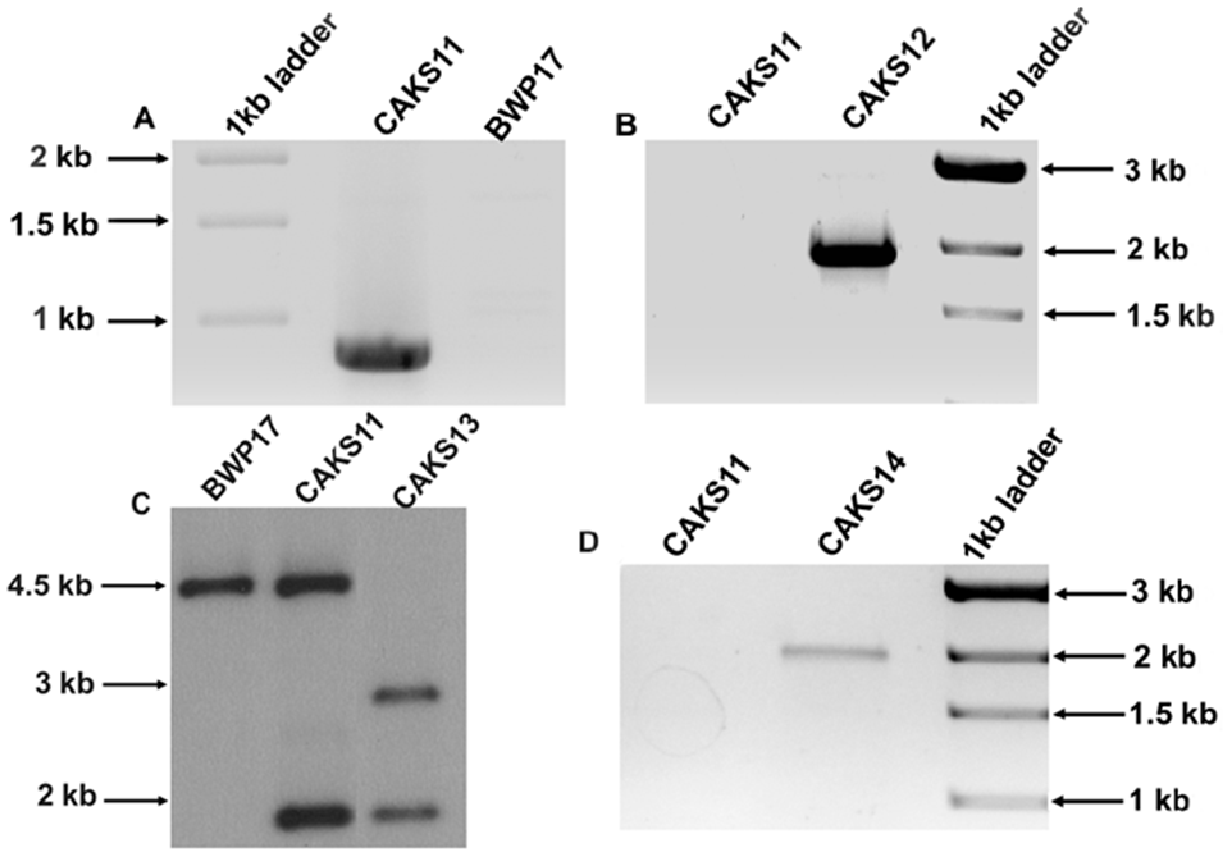
B



Supportng Figure S6



Supporting Figure S7



Supporting Figure S8

Figure S1. Construction of CAKS11, CAKS12, CAKS13, and CAKS14. Representative schematic of the diploid Ca*MTW1* loci in strains CAKS11, CAKS12, CAKS13 and CAKS14 is shown. CAKS11 and CAKS12 carry one disrupted copy and a full-length Ca*MTW1* allele. The full length Ca*MTW1* allele is under control of either its own promoter (CAKS11) or a regulatable *PCK1* promoter (CAKS12). CAKS13 contains one allele of *MTW1,* which is truncated and the second allele is tagged with the TAP epitope at its C-terminus. CAKS14 carries one truncated allele and one full length allele with a G71E mutation (ts*MTW1*).

Figure S2. CAKS14 cells arrested at G2/M phase of the cell cycle and showed chromosome segregation defects at 37°C. (A) CAKS14 cell viability dropped dramatically between 0.5 h and 2 h after a shift in incubation temperature from 23°C to 37°C. Cells were harvested at 30 min intervals after the temperature shift. Cell viability was calculated by plating diluted aliquots of culture at the indicated time points on YPDU plates and counting the number of colonies that appeared after incubating the plates for 3 days at 23°C. (B) Representative DIC images of BWP17 and CAKS14 cells, harvested at the indicated time points, after the temperature shift from 23°C to 37°C. After 1 h, CAKS14 cells started to accumulate at the large-budded stage followed by bud elongation (elongated bud phenotype or pseudohyphae-like terminal phenotype). (C) Distribution of unbudded (G1), small budded (S) and large budded (G2) cells of BWP17 and CAKS14 after temperature shift from 23°C to 37°C. (D) Table representing nuclear morphologies determined by DAPI staining of DNA of the BWP17 and CAKS14 cells harvested at 3 h after the temperature shift.

Figure S3. **CaMtw1 depleted cells and ts-mtw1 mutant (at 37° C) showed similar defects in the spindle morphology.** (A) BWP17 (*MTW1/MTW1*) and CAKS14 (*ts-mtw1/mtw1*) cells (grown at 37° C for 3h) were fixed and stained with anti-tubulin (spindle) antibodies and DAPI

(DNA). (Bar, 5μm). (B) Representative images of CAKS12 (*PCK1*pr-*MTW1/mtw1*) cells grown in glucose for 7 h at 30°C. These cells showed short mitotic spindle, improperly migrated to the daughter bud, sometimes leaving some unattached DNA in the mother bud (indicated by white arrows) (Bar, 5μm).

Figure S4. Confirmation of CaCse4 depletion. Western analysis with anti-CaCse4 antibodies was performed on cell lysates of YJB11483 and YJB10695, grown overnight in succinate media and then transferred to glucose media and incubated for 7 h. Ponceau s staining of the membrane shows equivalent protein loading.

Figure S5: GFP signal intensity measurement in CaMtw1-GFP, CaCse4-GFP, CaMif2-GFP strains. (A) Average Mtw1-GFP signal intensity in the kinetochore region was measured in YJB11482 (*CSE4/cse4*) (grown in glucose media) and YJB11483 (*PCK1*pr-*CSE4/cse4*) in depletion (glucose) and overexpression (succinate) conditions for the *PCK1* promoter. For each strains and condition, the average pixel intensity, corrected for background, was determined for at least 100 cells and is shown as mean ± SEM. * indicates that signal intensity was significantly different than YJB11482 ($p<0.01$ by one-way ANOVA and Bonferroni post-tests). (B) Average Cse4p-GFP signal intensity in the kinetochore region was measured in YJB11553 (*MTW1/mtw1*) and YJB11554 (*PCK1*pr-*MTW1/mtw1*) in depletion (glucose) and overexpression (succinate) conditions for the *PCK1* promoter. For all strains and conditions, the average pixel intensity, corrected for background for at least 100 cells per condition, is shown as mean ± SEM. * indicates that signal intensity was significantly different than YJB11482 ($p<0.01$ by one-way ANOVA and Bonferroni post-tests). (C) Average Mif2-GFP signal intensity in the kinetochore region was measured in YJB12118 (*MTW1/mtw1*) and YJB12119 (*PCK1*pr-*MTW1/mtw1*) in depletion (glucose) and overexpression (succinate) conditions for the *PCK1* promoter. For all

strains and conditions, the average pixel intensity, corrected for background for at least 100 cells per condition, is shown as mean ± SEM. * indicates that signal intensity was significantly different than YJB12118 ($p<0.0001$) by one-way ANOVA and Bonferroni post-tests).

Figure S6. Overexpression of *CaCSE4* results into higher recruitment of CaCse4 and CaMtw1 at centromere DNA. (A) ChIP analysis with anti-CaCse4 antibody in YJB11483 and YJB11482 grown in succinate media (where Ca*CSE4* is overexpressed from the *PCK1* promoter in YJB11483) showed increased binding of CaCse4 to the centromere of chromosome 5. (B) ChIP analysis with anti-GFP antibody in the same strains grown under the same conditions revealed increased recruitment of CaMtw1-GFP across the chromosome 5 centromere.

Figure S7. Identification of Ca*MTW1*: The CaMtw1 sequence was identified by a BLAST search with *S. cerevisiae* ScMtw1 as a query sequence against the *Candida albicans* genome database. This sequence analysis suggests that ORF19.1367 has significant homology to ScMtw1. A pairwise sequence comparison of ScMtw1 and CaMtw1 revealed that they share overall 29% identity. The homologous regions are restricted to two blocks localized at the N-terminus of the protein sequence (9-48th and 67-102nd amino acids of CaMtw1).

Figure S8. Confirmation of the construction of the strains CAKS11, CAKS12, CAKS13 and CAKS14. (A) PCR with genomic DNA of CAKS11 and BWP17 using MTW1-15 and MTW1-17 primers amplified the desired 870 bp DNA fragment in the case of CAKS11 but not in BWP17. (B) PCR with CAKS11 and CAKS12 genomic DNA using MTW1-8 and CA26 primers resulted in amplification of 2 kb DNA fragment in case of CAKS12, indicating that integration of pr. *PCK1-MTW1* occurred at the correct locus of the genome. (C) Genomic DNA samples of BWP17, CAKS11, and CAKS13 were digested with *Afl*III, blotted to Zeta-probe membrane (Millipore) and probed with an 805 bp *MTW1* promoter sequence. Lane1. BWP17 yielded a

single predicted ~4.5 kb band, lane 2: CAKS11 gave an additional, deletion-specific ~2 kb band, and lane 3: CAKS13 yielded a deletion-specific and one *MTW1*-TAP integration specific ~3 kb band. (D) PCR amplification with CAKS11 and CAKS14 genomic DNA using CAMA2con and Cal2 primers resulted in amplification of a 2056 bp fragment DNA in case of CAKS14, confirming that ts*MTW1* cassette has integrated into the correct locus of the genome.

References:

Joglekar, A. P., Bouck, D., Finley, K., Liu, X., Wan, Y., Berman, J. *et al*., (2008). Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres. *J Cell Biol*. **181**:587-94.

Wilson, R. B., Davis, D., Mitchell, A. P. (1999). Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J Bacteriol*. **181**:1868-74.

Table S1: List of strains of *C. albicans*:

Strain name	Genotype	Source
BWP17	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG*	Wilson *et al*. 1999
CAKS11	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG,*	This study

	MTW1/mtw1::HIS1	
CAKS12	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, mtw1*::*PCK1*pr-*MTW1(URA3)/mtw1*::*HIS1*	This study
CAKS13	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, mtw1*::*MTW1*-TAP(*URA3)/mtw1*::*HIS1*	This study
CAKS14	Δ*ura3::imm434*/Δ*ura3::imm434* Δ*his1::hisG*/*his1::hisG* Δ*arg4::hisG*/Δ*arg4::hisG, mtw1::ts-mtw1(URA3)/mtw1::HIS1*	This study
YJB10695	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, MTW1/MTW1*-GFP (*NAT*)	Joglekar *et al*. 2008
YJB11482	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, CSE4/cse4*::*hisG, MTW1/MTW1*-GFP(*NAT)*	This study
YJB11483	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, cse4*::*PCK1*pr-*CSE4(URA3)/cse4*::*hisG, MTW1/MTW1*-GFP*(NAT)*	This study
YJB10704	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, MTW1*-GFP*(URA3)/MTW1*-GFP*(NAT)*	Joglekar *et al*. 2008
YJB8675	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, CSE4/CSE4*-GFP-*CSE4*	Joglekar *et al*. 2008
YJB11553	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, mtw1::HIS1/MTW1, CSE4/CSE4*-GFP-*CSE4*	This study
YJB11554	Δ*ura3*::*imm434*/Δ*ura3*::*imm434* Δ*his1*::*hisG*/Δ*his1*::*hisG* Δ*arg4*::*hisG*/Δ*arg4*::*hisG, mtw1*::*PCK1*pr-*MTW1(URA3)/mtw1*::*HIS1, CSE4/CSE4*-GFP-*CSE4*	This study
YJB12118	Δ*ura3::λimm434/* Δ*ura3::λimm434* Δ*his1::hisG*/Δ*his1::hisG* Δ*arg4::hisG*/Δ*arg4::hisG* Δ*mtw1::HIS1/MTW1, MIF2/MIF2-GFP-NAT1*	This study
YJB12119	Δ*ura3::λimm434/* Δ*ura3::λimm434* Δ*his1::hisG*/Δ*his1::hisG* Δ*arg4::hisG*/Δ*arg4::hisG* Δ*mtw1::HIS1/ mtw1*::*PCK1*pr-*MTW1(URA3, MIF2/MIF2-GFP-NAT1*	This study
YJB12176	Δ*ura3::imm434*/Δ*ura3::imm434* Δ*his1::hisG/his1::hisG* Δ*arg4::hisG*/Δ*arg4::hisG,*	This study

	mtw1::ts-mtw1(URA3)/mtw1::HIS1	

Table S2: Primers used in this study:

Primer name	Sequence
MTW1-1	GCCATTGATAATGATTTCAAGAAAATAGATTTTGC
MTW1-2	AATTTGAGCATTTATATTCTTTATTGCATCCACATC
MTW1-3	GCGAATTCTAATTTACCACACGACAGCAATGAAGTTGG
MTW1-4-1	GCAAGCTTCCGTCGATTAGTATTGATCTATTTTTG
MTW1-5	GCGGAGCTCCAATAAGAGAAGGAGAGAACTGGCATC
MTW1-6	GCGGATCCGTACAAGATGAAGTTGTTAAGAATAGCC
MTW1-7	CCGAAGCTTCAAGGCAGATCTCTACAGATGATGATTGC
MTW1-8	CCGGATCCGCAAAATCTATTTTCTTGAAATCATTATCAA TGGC
MTW1-9	GGGATATTTACCAAACTTACTGAAGATGAA
MTW1-10	GATTCTAATTTCCCCATCTCAATTTCTATT
MTW1-15	GAAGCAAGAAAGCTCCTCATGTCG
MTW1-17	CTAACTTCTGTCTCCTCATCCTCC
Cal2	CATGAAGCTTAATTTGAGCATTTATATTCTTTATTGC
Cal6	AACTGCAGCGAAGTTGTAATTCTTGCATG

Cal9	GGACTAGTATGTCAGATAAAACTTTAGACGA
Cal16-2	CCCAAGCTTTTTACCACACGACAGCAATGA
Cal17	CCGCTCGAGAGAACCTTTACAATCACCTAA
CA26	GCGGATCCACTGTATTCCAATTTAAC
CAMA2con	AATTTGAGCATTTATATTCTTTATTGC
2498-15	CAAGCTGCCTTGTCAGGCAAAGCATC
2498-16	CCATCTCCAACCCGCCATGCCAGC
2498-23	GTATGACCTAAAGCTGTGAGCTGC
2498-24	CAGAGCAATGGCCCTTGTGATTGT
2498-9	GAAACGATCCTTCCTGTACACCAC
2498-10	CTTGATAGCGATCAGTGGGTTCAG
2498-21	CTAGTGCAAGACCCTCATAGAAGC
2498-22	CCTGACACTGTCGTTTCCCATAGC
2498-7	GCCTGTAGCGATGTAAGTATATGGAG
2498-8	CCACCTCTGCACTAATCTACAATGC
2498-5	GCGTAACGGGCCTAGTTTCGATAAGAG
2498-6	CATGCACAGGCTCTTATAGCAAGT
2498-19	GCCATACGGTAGTCAAACTCCTGG
2498-20	CCTGAACCACTACTGCAGAAACGT
2498-17	GCTTGGCCCTCAGTATAACTGGAT
2498-18	CTTCAGGACAAGCTCCATATCTCTTC
2498-1	CACTAACCCCATCTGAAACAAAGCG
2498-2	GGTCAGCACAATAATCACTGCACCAAC

CALEU2-1	GTGACCATGTCGGTACCGAAATTGTC
CALEU2-2	CTTGTTCAGGACGAACAGTGCCAGTA
nCEN7-3	GCATACCTGACACTGTCGTT
nCEN7-4	AACGGTGCTACGTTTTTTTA
nLeu2-1	GTACCGAAATTGTCAATGAAG
nLeu2-2	GTGGTGTTTGAAATCAAATTG
CACH5R1	TTCATGGAAGAGGGGTTTCA
CACH5F1	CCCGCAAATAAGCAAACACT
JB658	TTTGTACAATTCATCCATACCATG
JB2715	CATTGATATGATAATACCAGAACAAGACGATATAGATG TGGATGCAATAAAGAATATAAATGCTCAAATTTCTAAA GGTGAAGAATTATT
JB2717	GCTAGTGGTGGTGGTGGTAATGAAG
JB2759	GATACTCAATCTGAAGAACCAAAATTAGTTTTACTTTAT GGAAGATTACATGAAAGATGGCATCAAGCAGGTAAAAC GACGGCCAGTGAATTC
JB3551	GCTGCTACCCAACAAGGATT
JB3924 (CEN5 C4)	GTGAGAAGAAAAGTAAATGACTTCGAT
JB3925 (CEN5 C4)	AAACACTTGCAACCAATACAGG
JB3993 (CEN7 C3)	CATGACTACTACCCCATAGGCTTT
JB3994 (CEN7 C3)	TGAAGAGGTTGGTGGTTTTGT
JB4165 (LEU2)	TTTGGTCTTTATGAGCCTTGC
JB4166 (LEU2)	CAAGATTGTAGCAATTGGATTCAC

JB3912 (CEN5 L1)	ACTTTCCCTATCTGATGTTGCAC
JB3913 (CEN5 L1)	TTTGTCAGGTCATCATCATTTCTT
JB3876 (CEN5 IR1)	TCTAGTGGGCTATTGTCTGTGG
JB3877 (CEN5 IR1)	CGCAAGAATTTTGTTAACTTTGTG
JB3878 (CEN5 IR3)	CCAACTGAAACAAAATTTTCCAC
JB3879 (CEN5 IR3)	TGAGACAATGCTGCTAACGAG
JB3922 (CEN5 C2)	CCCTCTGTTGCTGTTACTTGAG
JB3923 (CEN5 C2)	TTTTGTATAAAGCAAGGCATTGAA
JB3880 (CEN5 C3)	TCATACACTAGCCTGTGCTCCTA
JB3881 (CEN5 C3)	TGATCGCATGAGAGAGTTGG
JB3926 (CEN5 C5)	AGGTGATTGTTGCATAGTCATTTC
JB3927 (CEN5 C5)	GGCTATAACTTCTAACTGGCATTGT
JB3933 (CEN5 R1)	CCAGCATCATCAGGCTCTTTA
JB3934 (CEN5 R1)	TGACAGAGATAGGATGCGTTATG
JB4674	TATTGGCCAAGGTGATGCTTATTTGTTTTTCGTTCAAAT CAGAAAACCAGAAGAAATCGATACCAATTGGGGTGGTG GTTCTAAAGGTGAAGAATTATT
JB4675	GACAAGGGTTATTAACTCGTGAACCTTCAAACAAAATG TAGCATTTAAATACTCGGCACAGTTGTAAACAGTAAAA CGACGGCCAGTGAATTC
p2488-1	CACTCTGACCAAATTCTCGTTTCC
p2488-2	GCAACATCCGAGTAAGGTTTGTGG
CACH2F	ACTGCTGGGCTTGTGAAGTT

CACH2R	GAGTCACAGCCAAACACGAA
CACH3F	AGATATGACGGCGCTGTTG
CACH3R	CAAACATCAACCTCCCCAAT
FCaCEN4	AGTACTTCATACAATCTTGGG
RCaCEN4	GGAAGAGTATGGTGTAG
CEN5-5F	TACTTCTGGTCAACGAGGCT
CEN5-5R	CCAATACAGGTTCCAATATG