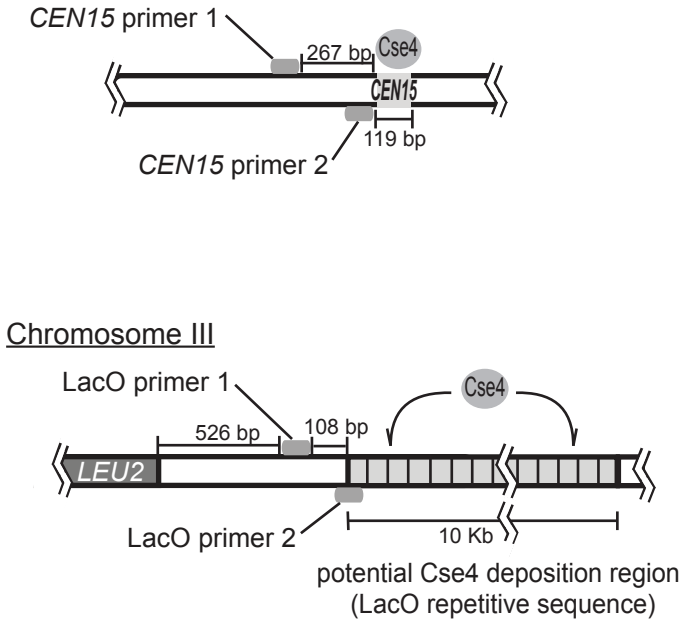
**Supplemental Information**

"Localization and function of budding yeast CENP-A depends upon kinetochore protein interactions and is independent of canonical centromere DNA sequence"

by Kung-Hsien Ho, Dai Tsuchiya, Audrey C. Oliger, and Soni Lacefield*

Supplemental Figure S1.

A.




B.

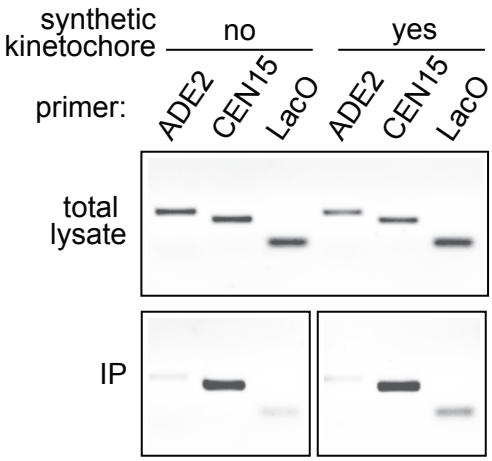


Figure S2

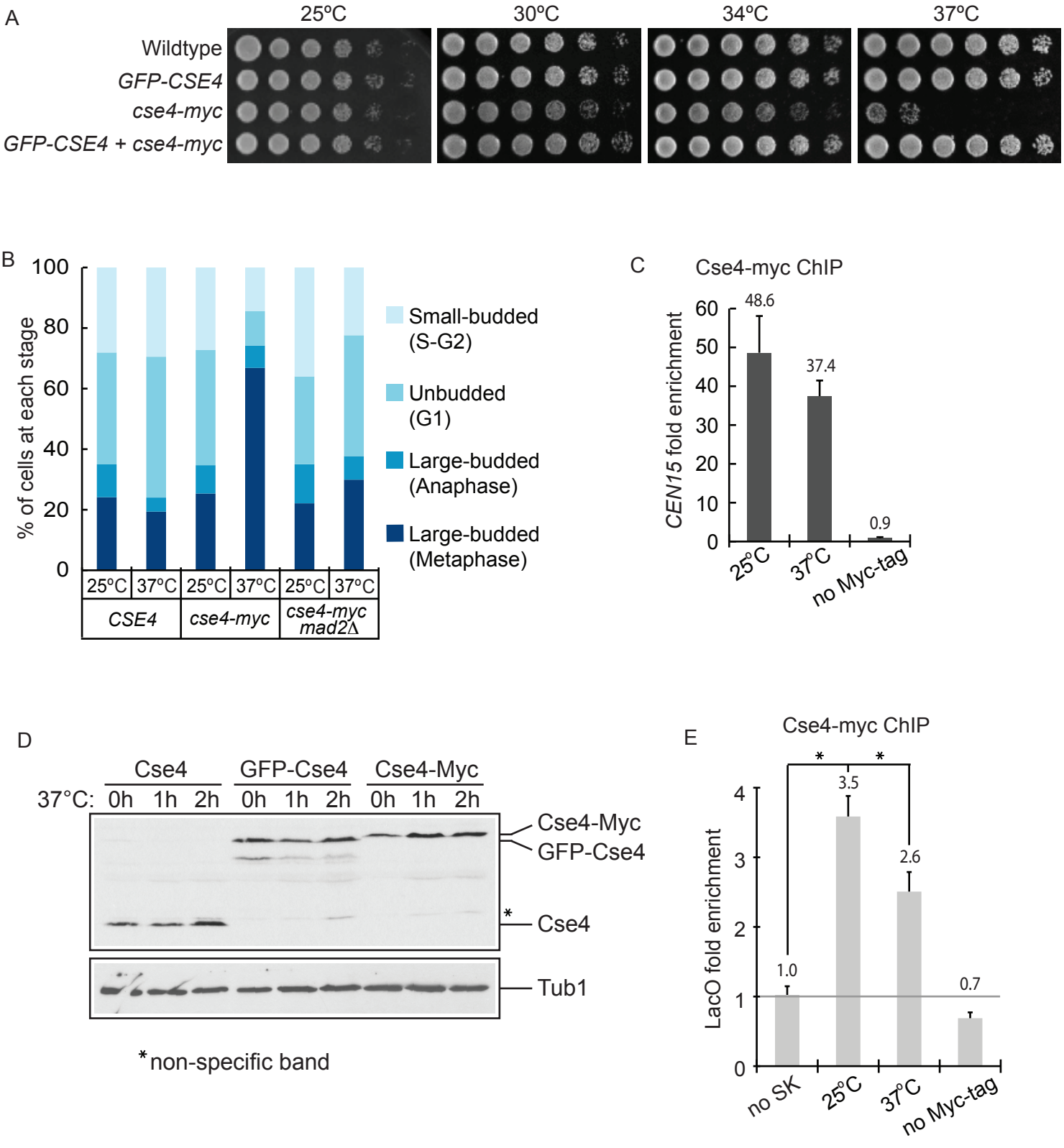
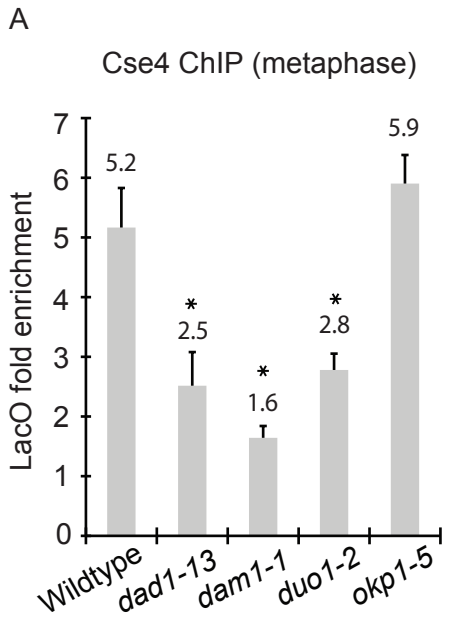
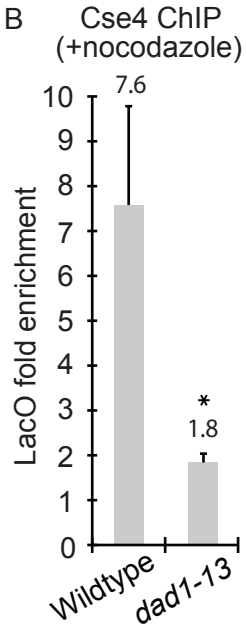
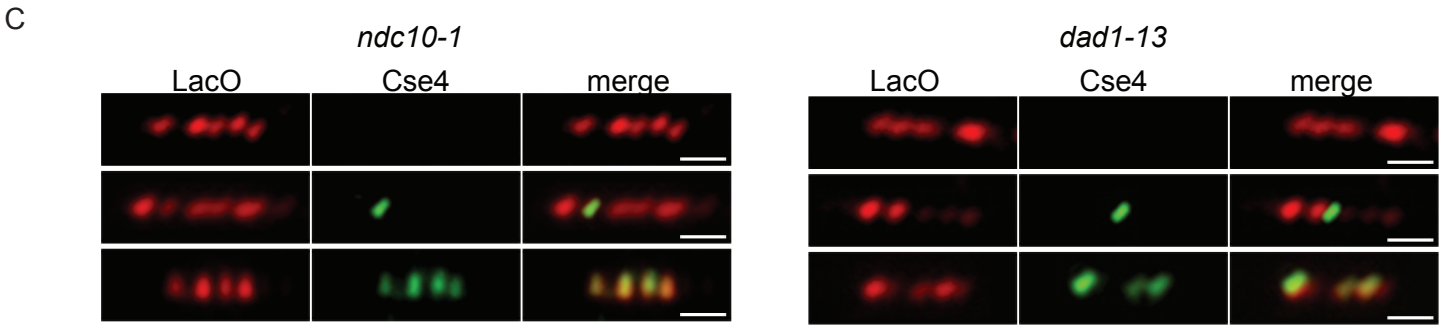

A
25ºC
30ºC
34ºC
37ºC
Wildtype
GFP-CSE4
cse4-myc
GFP-CSE4 + cse4-myc
B
% of cells at each stage
100
80
60
40
20
0
25°C 37°C 25°C 37°C 25°C 37°C
CSE4
cse4-myc
cse4-myc mad2Δ
Small-budded (S-G2)
Unbudded (G1)
Large-budded (Anaphase)
Large-budded (Metaphase)
C
Cse4-myc ChIP
CEN15 fold enrichment
60
50
40
30
20
10
0
48.6
37.4
0.9
25°C
37°C
no Myc-tag
D
Cse4
GFP-Cse4
Cse4-Myc
37°C: 0h 1h 2h 0h 1h 2h 0h 1h 2h
Cse4-Myc
GFP-Cse4
*
Cse4
Tub1
*non-specific band
E
Cse4-myc ChIP
LacO fold enrichment
4
3
2
1
0
*
*
3.5
2.6
1.0
0.7
no SK
25°C
37°C
no Myc-tag

Figure S3

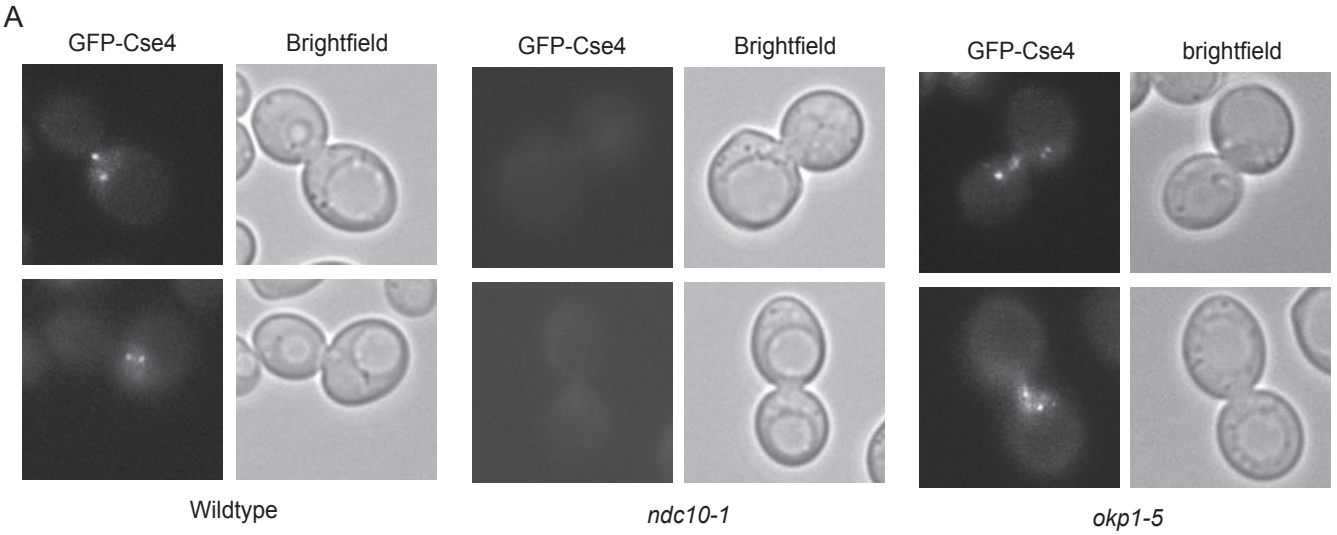


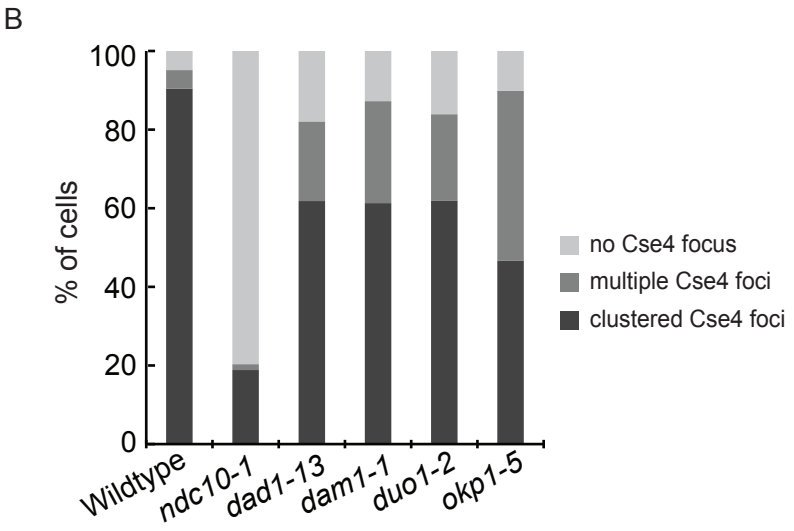




| | Wildtype no synthetic kinetochore | Wildtype with synthetic kinetochore | *ndc10-1* | *dad1-13* |
|---|---|---|---|---|
| No Cse4 on LacO | 94% | 49% | 74% | 73% |
| One Cse4 focus on LacO | 5% | 27% | 16% | 20% |
| Multiple Cse4 foci on LacO | 1% | 24% | 10% | 7% |

Figure S4


A
GFP-Cse4
Brightfield
GFP-Cse4
Brightfield
GFP-Cse4
brightfield
Wildtype
ndc10-1
okp1-5


B
100
80
60
40
20
0
% of cells
no Cse4 focus
multiple Cse4 foci
clustered Cse4 foci
Wildtype
ndc10-1
dad1-13
dam1-1
duo1-2
okp1-5

**Supplemental Figure Legends:**

**Figure S1, related to Figure 1) ChIP assay using anti-GFP antibody to IP GFP-Cse4.** A) Diagram of the location of the oligos used in the ChIP-qPCR to amplify a unique region within LacO. B) Three loci, *ADE2, CEN15*, and LacO were tested for their presence in the total lysate and IP fractions in strains with and without the synthetic kinetochore. The amplified DNA was run out on a 1% agarose gel.

**Figure S2, related to Figure 2) *Cse4-Myc* is a loss-of-function mutant allele of *CSE4*.** A) 1:5 dilutions of saturated cultures spotted onto rich medium plates and grown at 25, 30, 34, and 37°C, showing that *cse4-myc* cells are temperature sensitive and *GFP-CSE4* can rescue the temperature sensitivity. B) Quantification of the percent of cells at each cell-cycle stage following alpha-factor arrest and then release at 25 or 37°C. Cells were stained with DAPI and nuclear segregation was analyzed to determine the percent of anaphase versus metaphase cells. The bar graph represents the average of 3 experiments with 100 cells counted per experiment. The *cse4-Myc* cells are enriched at metaphase at 37°C and this enrichment is dependent on the spindle checkpoint protein Mad2. C) The ChIP of Cse4-Myc with anti-Myc antibody, comparing cells grown at 25 and 37°C. The quantification of ChIP-qPCR results from at least three independent experiments. The amount of *CEN15* from the IP is normalized to the amount of *ADE2*. The no Myc-tag control

shown is from cells incubated at 37°C, but cells incubated at 25°C gave a similar result. Error bars represent the standard deviation. D) Immunoblots using anti-Cse4 and anti-Tub1 antibodies from protein isolated from *CSE4, GFP-CSE4,* and *cse4-Myc* cells. * denotes non-specific bands. E) The ChIP of Cse4-Myc with anti-Myc antibody, comparing cells grown at 25 and 37°C. The quantification of ChIP-qPCR results from at least three independent experiments. The amount of LacO from the IP is normalized to the amount of *ADE2*. The no Myc-tag control shown is from cells grown at 37°C, but the result at 25°C was similar. Error bars represent the standard deviation.

**Figure S3, related to Figure 3) Dam-DASH mutant cells arrested in metaphase have decreased levels of Cse4 at LacO compared to cells with wildtype kinetochore proteins.** A, B) The ChIP of GFP-Cse4 with anti-GFP antibody, comparing cells with kinetochore temperature-sensitive mutations. The bar graph shows the quantification of ChIP-qPCR results from at least three independent experiments. The amount of LacO from the IP is normalized to the amount of *ADE2*. Error bars represent the standard deviation. The * represents statistical significance (t test, $p < .05$). Cells were incubated at 37°C for two hours prior to the ChIP. A) Cells were arrested in metaphase due to depletion of Cdc20. B) Cells were treated with nocodazole and benomyl to disrupt kinetochore-microtubule interactions. C) Spread chromatin fibers from *ndc10-1* and *dad1-13* cells showing no Cse4

on the Lac O array, one Cse4 focus on the LacO array, and multiple Cse4 foci on the LacO array, respectively. Percent of cells in each category are shown below.

**Figure S4, related to Figure 4) Assay of centromere clustering in kinetochore temperature-sensitive mutants.** Cells with different kinetochore temperature-sensitive mutations were grown with the conditions used for ChIP. Cells were monitored for GFP-Cse4 clusters. A) Representative images from metaphase wildtype, *ndc10-1*, and *okp1-5* mutant cells grown at the restrictive temperature. Images show clustered Cse4 foci (wildtype cells), no Cse4 foci (*ndc10-1* cells), and multiple Cse4 foci (*okp1-5* cells). B) Counts of GFP-Cse4 clusters in kinetochore temperature sensitive mutations grown at the restrictive temperature. Data shown represent the average of three experiments with 100 cells counted per experiment per genotype.

## Supplemental Tables

### Supplemental Table 1:

| Strain | Genotype |
|---|---|
| LY531 | *MATa CSE4-myc13:KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, LacO256:TRP1-CHRIV$_{800K}$, ade2-1, leu2-3,112, trp1-1, ura3-1, bar1-1, can1-100* |
| LY738 | *MATa $P_{HIS3}$-Ask1-LacI(I12):HIS3, LacO256:TRP1-CHRIV$_{800K}$, ade2-1, leu2-3,112, trp1-1, ura3-1, can1-100* |
| LY755 | *MAT$\alpha$ $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, ura3-1, can1-100* |
| LY844 | *MATa CSE4-myc13:KanMX6, $P_{HIS3}$-GFP-LacI(I12):ADE2, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, URA3-CHRIII$_{116K}$, $P_{MET3}$-HA3-CDC20:TRP1, trp1-1, ura3-1, can1-100* |
| LY852 | *MATa CSE4-myc13:KanMX6, $P_{CUP1}$-GFP-LacI(I12):HIS3, LacO256-CEN15:URA3, $P_{MET3}$-HA3-CDC20:TRP1, Pds1-myc18:LEU2, ade2-1, leu2-3,112, trp1-1, ura3-1, can1-100* |
| LY1308 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, his3-11,15, trp1-1, can1-100* |
| LY1253 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ndc10-1, ade2-1, trp1-1, can1-100* |
| LY1254 | *MAT$\alpha$ $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, dad1-13, ade2-1, trp1-1, can1-100* |
| LY1255 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, dam1-1, ade2-1, trp1-1, can1-100* |
| LY1308 | *MAT$\alpha$ $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, his3-11,15, trp1-1, can1-100* |

| | |
|---|---|
| LY1309 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100* |
| LY1323 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, okp1-5:TRP1, ade2-1, trp1-1, can1-100* |
| LY1354 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, nsl1-5, ade2-1, trp1-1, can1-100* |
| LY1355 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, mtw1-11, ade2-1, trp1-1, can1-100* |
| LY1356 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ndc80-1, ade2-1, trp1-1, can1-100* |
| LY1357 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, nuf2-61, ade2-1, trp1-1, can1-100* |
| LY1358 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, spc25-1, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100* |
| LY1361 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, duo1-2, ade2-1, trp1-1, can1-100* |
| LY1367 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, dsn1-7, ade2-1, trp1-1, can1-100* |
| LY1368 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, nnf1-77, ade2-1, trp1-1, can1-100* |
| LY1436 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, mif2-3, ade2-1, trp1-1, can1-100* |

| | |
|---|---|
| LY1439 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ctf13-30, ade2-1, trp1-1, can1-100* |
| LY1440 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, spc24-1, ade2-1, trp1-1, can1-100* |
| LY1454 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ctf19D::KanMX6, ade2-1, trp1-1, can1-100* |
| LY1455 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, cnn1D::KanMX6, ade2-1, trp1-1, can1-100* |
| LY1456 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, psh1D::KanMX6, ade2-1, trp1-1, can1-100* |
| LY1477 | *MATa CSE4-myc13:KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, URA3-CHRIII$_{116K}$, ade2-1, trp1-1, ura3-1, can1-100* |
| LY1478 | *MATa CSE4-myc13:KanMX6, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, his3-11,15, trp1-1, URA3-1, can1-100* |
| LY1483 | *MATa Ndc10-GFP-:KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, ura3-1, can1-100* |
| LY1484 | *MATa Ndc10-GFP-:KanMX6, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, his3-11,15, trp1-1, ura3-1, can1-100* |
| LY1550 | *MAT$\alpha$ $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, his3 11,15, trp1-1, can1-100 + pLB86($P_{URA3}$-mCherry-LacI(wt):ADE2)* |
| LY1551 | *MAT$\alpha$ $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, , $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100 + pLB86($P_{URA3}$-mCherry-LacI(wt):ADE2)* |
| LY1669 | *MAT$\alpha$ CSE4-myc13:KanMX6, $P_{CSE4}$-GFP-Cse4:URA3, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100* |

| | |
|---|---|
| LY1774 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, $P_{MET3}$-HA3-CDC20:TRP1, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100* |
| LY1876 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(wt):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ade2-1, trp1-1, can1-100* |
| LY2066 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, dad1-13, ade2-1, trp1-1, can1-100 + pLB86($P_{URA3}$-mCherry-LacI(wt):ADE2)* |
| LY2076 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, $P_{MET3}$-HA3-CDC20:TRP1, ura3-CHRIII$_{116K}$, duo1-2, ade2-1, trp1-1, can1-100* |
| LY2078 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, $P_{MET3}$-HA3-CDC20:TRP1, ura3-CHRIII$_{116K}$, okp1-5:TRP1, ade2-1, trp1-1, can1-100* |
| LY2079 | *MATα $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, $P_{MET3}$-HA3-CDC20:TRP1, ura3-CHRIII$_{116K}$, dad1-13, ade2-1, trp1-1, can1-100* |
| LY2080 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, $P_{MET3}$-HA3-CDC20:TRP1, ura3-CHRIII$_{116K}$, dam1-1, ade2-1, trp1-1, can1-100* |
| LY2113 | *MATa $P_{CSE4}$-GFP-Cse4:URA3, cse4::KanMX6, $P_{HIS3}$-Ask1-LacI(I12):HIS3, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, ura3-CHRIII$_{116K}$, ndc10-1, ade2-1, trp1-1, can1-100 + pLB86($P_{URA3}$-mCherry-LacI(wt):ADE2)* |
| SLY834 | *MATa $P_{HIS3}$-GFP-LacI(I12):ADE2, $P_{GAL1}$-CEN3:TRP1, LacO256:LEU2, URA3-CHRIII$_{116K}$, $P_{MET3}$-HA3-CDC20:TRP1, his3-11,15, trp1-1, ura3-1, can1-100* |

| | |
|---|---|
| SLY835 | *MATa* $P_{HIS3}$*-GFP-LacI(I12):ADE2,* $P_{HIS3}$*-Ask1-LacI(I12):HIS3,* $P_{GAL1}$*-CEN3:TRP1, LacO256:LEU2, URA3-CHRIII*$_{116K}$*,* $P_{MET3}$*-HA3-CDC20:TRP1, trp1-1, ura3-1, can1-100* |
| SLY904 | *MATa* $P_{CUP1}$*-GFP-LacI(I12):HIS3, LacO256-CEN15:URA3,* $P_{MET3}$*-HA3-CDC20:TRP1, Pds1-myc18:LEU2, ade2-1, leu2-3,112, trp1-1, ura3-1, can1-100* |

**Supplemental Table 2.**

| **Oligo description** | **Sequence** |
|---|---|
| LacO ChIP | TGGAATTCTCGAGGGATCCCC |
| | CTGCAAGGCGATTAAGTTGGG |
| Ade2 ChIP | GCCGGTAATGTCTGCCGCATGTGCGG |
| | TCTGACAGCCAACAGCGCAGCGTT |
| CEN15 ChIP | CATTCGCCATATCCGCAAGTTTGTC |
| | CCCAAAAATATGAGGGCATCGG |

**Supplemental Table Legend:**

**Supplemental Table 1: Strains used in this study, related to Figures 1-4**

**Supplemental Table 2: Oligos used for ChIP in this study, related to Figures 1-4.**

**Supplemental Experimental Procedures:**

*Strains, plasmids, and plating assays.* Strains are derivatives of W303 and are listed in Supplemental Table 1. Deletion and tagged strains were made using standard methods (Longtine et al., 1998), unless described below. The *CSE4*-promoted-*GFP-CSE4* construct, with GFP integrated at residue 83 with 13 and 18-mer linkers at each end, was integrated to the *URA3* locus and the endogenous *CSE4* was deleted (Chen et al., 2000). The C-terminally tagged *CSE4-13Myc* was made through PCR integration of the 13Myc epitope (Longtine et al., 1998). Chromosomes were tagged with the LacO array, Ask1-LacI, and GFP-LacI as described (Lacefield et al., 2009; Straight et al., 1996). The LacI in the Ask1-LacI and GFP-LacI fusion proteins have two mutations: a mutation that increases binding affinity for LacO (LacI-I12), and a deletion of the tetramerizing domain to prevent LacI from linking sister chromatids together (Schmitz et al., 1978; Straight et al., 1996). The mCherry-LacI was generated by replacing GFP in the plasmid containing GFP-LacI with a mCherry construct. For the spotting assays, saturated cultures were diluted serially by 1/5 and spotted onto appropriate plates and incubated at 25, 30, or 37$^{o}$C for 2-3 days.

*Chromatin Immunoprecipitation (ChIP).* ChIP was performed as described, with the modifications listed below (Strahl-Bolsinger et al., 1997). To turn off the natural *CEN* and

allow the full assembly of the synthetic kinetochore, cells were grown to logarithmic phase (1~5*$10^7$ cells/mL) in YEPRaf (YEP with 2% raffinose) at 25˚C and then 2% Galactose was added for one hour to activate the *GAL1,10* promoter at *CEN3*. The culture was then incubated at 25 or 37˚C for 2 hours and fixed with 1% formaldehyde. For nocodazole treatment, cells were incubated with 2% galactose at 25˚C for one hour to activate the *GAL1,10* promoter, switched to 37˚C for one hour, and then treated with 30 μg/mL benomyl (Sigma) and 15 μg/mL nocodazole (Acros Organic) for one hour at 37˚C to depolymerize the microtubules. For metaphase-arrest ChIP experiments, a *MET25* promoter was integrated to replace the endogenous promoter of *CDC20* for depletion of Cdc20 in media containing methionine. Cells were cultured to mid-log phase in –met media containing 2% raffinose at 25°C and switched to 37˚C with 2% galactose for one and half hours. Then, an equal volume of YEP with 2% galactose and raffinose was added and incubated at 37˚C for two more hours to reach metaphase arrest. Cell lysate was prepared by bead beating (0.5mm) in lysis buffer (50 mM HEPES-K pH 7.5, 140mM NaCl, 1% TX-100, 0.1% Na-Deoxycholate, 1mM PMSF, 1μg/mL pepstatin A, proteinase inhibitor cocktail (complete, Mini, Roche)) for 30 minutes at 4˚C. Isolated DNA was sheared to 400~800 base pairs using a sonicator (Heat systems-Ultrasonics, inc, model W-225, setting 2.5, 10 seconds 4 times). Cell debris was removed by centrifugation. 50μL of the purified DNA was reserved as total

lysate. The remaining sample was incubated with 5.4µg anti-GFP (Life Technologies) or 3.5µg anti-myc (Roche) antibodies at 4˚C for 2 hours, and then incubated with 0.84mg Protein G-coupled dynabeads (Life Technology) at 4˚C for 2 hours. The dynabeads were washed five times and eluted with 250µL elution buffer (50mM Tris-Cl pH8.0, 10mM EDTA, 1% SDS). The eluate was incubated at 65˚C overnight, digested with 100µg Proteinase K for 2 hours, and extracted by phenol/chloroform into 30µL water.

*Quantitative PCR (qPCR).* Sequences of the oligos used for qPCR are listed in Suppl. Table 2. The qPCR was performed as described with the modifications listed (Haring et al., 2007). The purified total lysate DNA from the ChIP was diluted to 100 ng/µL and then serially diluted by 1/5 four times. The purified ChIP precipitated DNA was diluted to 0.3 ng/µL. The diluted DNA was then mixed with 200 nM primers (*ADE2*, LacO, and *CEN15*) and Brilliant III Ultra-Fast SYBR Green QPCR Master mix (Agilent). The qPCR reaction was conducted with the (Agilent Mx3005P) qPCR machine. The resulting Ct number of precipitated DNA was converted to a relative concentration using the standard curve built with the serial dilutions of the total lysate DNA. The quantity of precipitated ADE2 DNA from each strain represents non-specific binding and was used to normalize the quantity of LacO and *CEN15* DNA to show the increase of precipitated DNA over non-specific binding. The results in

each figure are the average of at least three repeats. The LacO primers were designed to amplify a region adjacent to the LacO array and not within the array to prevent the amplification of different numbers of repeats, which would interfere with the quantification.

The percent reduction of *CSE4* enrichment in the temperature-sensitive mutants was calculated relative to the specific recruitment to the synthetic kinetochore in comparison to the no synthetic kinetochore control. For example, in the *ndc10-1* mutant there was (1.6-0.9)/(3.9-0.9)*100 = 23% of the level in wildtype or 100-23% = 77% reduction from wildtype cells (Figure 3C).

*Chromatin fiber immunofluorescence.* Genomic chromatin fibers were prepared from protoplasts. Cells were grown to mid-log phase in synthetic complete media with 2% Raffinose (SCRaf), and then 2% galactose was added for 90 minutes. Cells were fixed with 1% paraformaldehyde for 1min and washed with phosphate-buffered saline (PBS). Cells were treated with 10 mM Dithiothreitol (DTT) buffered with 0.1M Tris-$SO_4$ (pH 9.4) for 30 min, and the cell wall was digested with 0.5 mg/ml zymolyase (Zymoresearch) buffered with 1M sorbitol. Harvested protoplasts were treated in NDS (0.5 M EDTA, 10 mM Tris-Cl, pH 8.0, 0.1% (w/v) sodium lauroyl sarcosinate) for 30 min. Extracted chromatin fibers

were mechanically spread on the positively charged glass slides using the edge of a cover slip. Slides were blocked with 5% Bovine Serum Albumin (Sigma) for 1h at 25°C, then incubated with primary antibodies, either Chicken anti-GFP (Novus, 1:600) or Rabbit anti-mCherry (Novus, 1:600), overnight at 4°C. Slides were washed twice with PBS, once with PBS containing 0.1% tween20, once with PBS. Secondary antibodies, Alexa 488 conjugated goat anti-chicken (Molecular probe, 1:200) or Alexa 594 conjugated goat anti-Rabbit (Molecular probe, 1:200) was applied. Slides were washed 3 times with PBS, and mounted with anti-fading media (Vectashield, Vector laboratories).

*Microscopy.* Images were acquired with an epifluorescence microscope (Nikon Ti-E) equipped with a 100X objective (Plan Apo, NA 1.49), a Lambda 10-3 optical filter changer and SmartShutter (Sutter Instrument, Novato, CA), GFP and mCherry filters (Chroma Technology, Bellows Falls, VT), and a CoolSNAP HQ2 charge-coupled device camera (Photometrics, Tucson, AZ).

*Cell cycle stage analysis.* Logarithmically growing cells in YPD at 25˚C were arrested with 100µM alpha-factor for two hours. The arrested cells were incubated at 25 or 37˚C for 1 hour and released from the arrest at 25 or 37˚C for 4 hours. Cells were then fixed with

100% methanol for microscopy analysis of morphology. The nucleus was stained with 1 μg/mL DAPI. The bar graph represents the average of 3 experiments, with at least 100 cells counted for each experiment.

*Biorientation Assay*. The biorientation assay was performed as described (Lacefield et al., 2009). Cells contain a *MET25* promoted *CDC20* to allow a metaphase arrest through the depletion of Cdc20 in media containing methionine. Cells were cultured to mid-log phase in –met media containing 2% raffinose at 25˚C, arrested with alpha-factor, and then incubated at 37˚C for 2 hours. Galactose (2%) was added to the culture for 2 hours to activate transcription at the *GAL1,10-CEN3*. Cells were then released into YPGal at 37˚C for 2 hours to reach metaphase arrest. For fluorescence microscopy, cells were fixed with 0.4% paraformaldehyde and analyzed for GFP foci. Cells with 2 separate GFP foci near the bud neck were considered bioriented. A total of 3 experiments were performed and averaged with at least 100 cells counted per experiment.

*Immunoblotting analysis*. The immunoblotting experiments were performed as described (Ho et al., 2009). Briefly, strains expressing Cse4, GFP-Cse4, or Cse4-Myc were grown to mid-log phase in YPD at 25°C and incubated at 37°C for 0, 1, or 2 hours. Approximately

1~2 * $10^5$ cells were harvested, fixed with 10% TCA, washed with acetone twice, and air-dried. The fixed cells were lysed in Laemmli sample buffer (125 mM Tris-Cl pH 6.8, 2% SDS, 15% glycerol, 0.005% bromophenol blue, 1% β-mercaptoethanol) by beat beating. The sample was boiled for 5 minutes and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% acrylamide gel. Resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, and blotted with anti-Gse4 or anti-Tub1 antibodies.

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