Ipl1/Aurora B kinase coordinates synaptonemal complex disassembly with cell cycle progression and crossover formation in budding yeast meiosis

Article (Unspecified)


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A. Tubulin dot + single DNA body

B. Short meta I spindle + 1 DNA body

C. Long meta I/early ana I + 1 DNA body

D. Anal + 2 DNA bodies

E. Short meta II spindles + 2 DNA bodies

F. Long meta II/early ana II + 2 DNA bodies

G. Anal II + 4 DNA bodies

Proportion of cells over Hrs in SPM for WT and ipf1-mn.
Wild type

ipl1-mn

zip1Δ

ipl1-mn, zip1Δ

A  1 nucleus

B  1 stretched nucleus

C  2 nuclei

D  2 stretched nuclei

E  4 nuclei

Proportion of cells

Proportion of cells

Proportion of cells

Hrs in SPM

Hrs in SPM

Hrs in SPM
A) Wild type (CLB3-13MYC)

Proportion of cells

<table>
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<tr>
<th>Hrs in SPM</th>
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<th>6</th>
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<td>1n - stretched</td>
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B) Wild type (CLB3-13MYC)

Proportion of cells

<table>
<thead>
<tr>
<th>Hrs in SPM</th>
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<th>6</th>
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<tbody>
<tr>
<td>&gt; 2n</td>
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<tr>
<td>2n - stretched</td>
<td></td>
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</tbody>
</table>

C) ipl1-mn (CLB3-13MYC)

Proportion of cells

<table>
<thead>
<tr>
<th>Hrs in SPM</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
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<tr>
<td>1n - stretched</td>
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</table>

D) ipl1-mn (CLB3-13MYC)

Proportion of cells

<table>
<thead>
<tr>
<th>Hrs in SPM</th>
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<th>3</th>
<th>6</th>
<th>9</th>
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</tr>
<tr>
<td>2n - stretched</td>
<td></td>
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</table>
Wild type

0 15 30 45 60 90 120
Min. after NDT80 ON

ipl1-mn

0 15 30 45 60 90 120
Min. after NDT80 ON

λ phosphatase
inhibitors

- + +
- - +
A

Xhol/EcoRI digest

P1 X E leu2 URA3 ARG4 leu2
P2 X his4 E E X E

leu2 his4

arginate

Hrs in SPM

PROBE (180 bp)

B

Wild type

Hrs in SPM

0 2 3 4 5 6 7 8 9 10 11 12 M

CO1

NCO

P2

ipl1-mn

Hrs in SPM

0 2 3 4 5 6 7 8 9 10 11 12 M

9.4

6.6

4.4

2.3

2.2

D

NCO

Hrs in SPM

0 2 3 4 5 6 7 8 9 10 11 12

Proportion of DNA

0.1

0.08

0.06

0.04

0.02

0.0

ipl1-mn

WT

E

CO

Hrs in SPM

0 2 3 4 5 6 7 8 9 10 11 12

Proportion of DNA

0.1

0.08

0.06

0.04

0.02

0.0

ipl1-mn

WT
A

**metaphase I**

<table>
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<tr>
<th>DNA</th>
<th>tubulin</th>
<th>Cdc14</th>
<th>DNA</th>
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</table>

WT

**ipl1-mn**

Nucleolar Cdc14-13Myc

19/20 16/16

---

B

**anaphase I**

<table>
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<th>DNA</th>
<th>tubulin</th>
<th>Cdc14</th>
<th>DNA</th>
</tr>
</thead>
</table>

WT

**ipl1-mn**

Nucleolar Cdc14-13Myc

0/9 0/25
**A**

Wild type

- Diploctene
- Metaphase I

**B**

ipl1-mn

- Metaphase I
- Anaphase I

**Legend**

- Zip1
- Smt3
- DNA
Wild type

ipl1mn

DNA    tub    Red1    tub    Red1

DNA    tub    Red1    tub    Red1
**Wild type**

A

**ipl1-mn**

D

---

B

<table>
<thead>
<tr>
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<th>Dip</th>
<th>Met I</th>
<th>Ana I</th>
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<td>0.6</td>
<td>0.4</td>
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<tr>
<td>Dotty</td>
<td>0.8</td>
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<td>Dot- lines</td>
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<tr>
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Stage

---

C

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<thead>
<tr>
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<th>Ana I</th>
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<tbody>
<tr>
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<td>0.4</td>
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Stage

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E

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Stage

---

F

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<th>Proportion</th>
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<th>Met I</th>
<th>Ana I</th>
</tr>
</thead>
</table>

Stage
A  Wild type

B  ipl1-mn
A

diplotene

metaphase I

anaphase I

B

H3-Ser10 → Ala

pachytene

diplotene

metaphase I
Supplementary Material.

Supplementary Figure legends.

Supplementary Figure S1. 3HA-Ipl1 protein levels and kinase activity during meiotic prophase. (A) Western blot analysis of 3HA-Ipl1 expressed from the CLB2 promoter. (B) 3HA-Ipl1 levels were quantified relative to Pgk1 (gray bars) and then normalized to the 0 hour time point (black bars). Ipl1 activity was measured by determining the phosphorylation of Ser10 Histone H3 compared to total cellular levels of histone H3 in wild type (C, Y1381) and ipl1-mn (D, Y1669).

Supplementary Figure S2. Nuclear divisions and spindle behaviour in ipl1-mn mutants suggest a metaphase-anaphase delay. Cells were assessed for nuclear as well as spindle morphology simultaneously to determine whether nuclear divisions had been decoupled from spindle morphology. Cells were divided into six categories, described as follows. Proportion of cells with a single DNA mass and a single focus of tubulin (A), representative of meiotic prophase cells, with a metaphase I spindle and a single DNA mass (B), representative of metaphase I, or a long metaphase I/early anaphase I spindle and a single DNA mass (C), representative of late metaphase I. In the wild type (Y940), < 5% of such DNA masses were ‘stretched’ at late metaphase I, whereas in ipl1-mn (Y1206), the majority displayed a ‘stretched’ phenotype. (D) Proportion of cells with two clearly separated DNA bodies and an anaphase I spindle, representative of cells having completed the metaphase I-anaphase I transition. (E) Cells with two, short metaphase I spindles, each
with a single body of DNA, representative of cells in metaphase II. (F) Proportion of cells with long metaphase II/early anaphase II spindles, each with a single body of DNA (late metaphase II). In the ipl1-mn mutant, the majority of these nuclei were stretched, as in (C). (G) Cells with two anaphase II spindles and four separate DNA bodies represented cells that had successfully completed both meiotic nuclear divisions. Arrows indicate spindles formed prior to meiotic entry. At least 200 cells were counted for each time point.

**Supplementary Figure S3.** Stretched nuclei are observed in zip1 and ipl1-mn zip1. Time course experiments of nuclear divisions and stretched nuclear phenotypes in wild-type (Y940), ipl1-mn (1206), zip1 (Y1530), and ipl1-mn zip1 (Y1658) mutants. At least 200 cells were counted for each time point.

**Supplementary Figure S4.** Meiotic nuclear divisions are delayed despite normal expression of Clb3-13Myc in ipl1-mn cells. (A and C) Proportion of ethanol-fixed cells containing a single nuclear body (1n), a stretched nuclear body (1n - stretched), or two distinct nuclear bodies (2n). (B and D) Proportion of cells with more than two nuclear bodies or containing two nuclear bodies of which at least one was stretched (2n – stretched). β-estradiol was added 6 hours after cells were transferred to SPM. Strains: wild type (Y1581), ipl1-mn (Y1582).

**Supplementary Figure S5.** Delayed SC disassembly in the ipl1-as5 mutant (Y1583) treated with 1-NA-PP1. (A) Examples of surface-spread nuclei at
various stages. Zip1 is given in green and tubulin (tub) in magenta. (B) SC disassembly and PC occurrence (C) in mock-treated cells and 1-NA-PP1 treated cells (D and E). Bars: 2 µm. More than 100 nuclei were inspected for each time point.

**Supplementary Figure S6.** Zip1 is a phosphoprotein. Western blot analysis of Zip1 shows two bands in both wild-type (A, Y1602) and *ipl1-mn* (B, Y1538). Both bands are present prior to Ndt80 expression (0 minutes) and after Ndt80 expression. (C) Zip1 protein mock-treated (lane 1) treated with λ phosphatase (lane 2), or treated with both λ phosphatase and inhibitor.

**Supplementary Figure S7.** Schematic representation of the *URA3-ARG4* ectopic recombination interval on Chromosome III (Allers and Lichten, 2001). *Xho*I digest of DNA yields the indicated sizes of parental molecules (P1 and P2), DSBs (DSB1 and DSB2) as well as crossovers (CO1 and CO2). Sequences flanking the insert at *LEU2* and the insert at *HIS4* are denoted by solid grey and dashed grey lines, respectively. The region recognized by the probe is shown in blue.

**Supplementary Figure S8.** Detection of crossover and noncrossover products at the *URA3-ARG4* interval on Chromosome III (Allers and Lichten, 2001). (A) *Xho*I and *EcoR*I digest of DNA, probed with *HIS4*-specific sequences, yields the indicated sizes of parental (P2), double-strand break (DSB2), crossover (CO1) as well as noncrossover recombinants (NCO). The region recognized by the probe, which is specific to *HIS4* sequences, is
shown in blue. M- marker. The sizes, in kilobases, are given adjacent to the
ipl1-mn blot. (B and C) Autoradiograms of typical wild-type (Y940) and ipl1-mn
(Y1206) meiotic time courses. (D and E) Quantification of NCO products (D)
and CO products (E).

Supplementary Figure S9. Cdc14-13Myc release from the nucleolus in
nuclei containing metaphase I or anaphase I spindles. Examples of nuclei at
metaphase I (A) and anaphase I (B). DNA is shown in blue, Cdc14-13Myc in
green, and tubulin (tub) in red. The proportion of nuclei with metaphase I (A)
spindle and nucleolar Cdc14-13Myc focus is shown to the right of the image
for wild type (Y1662) and ipl1-mn (Y1664). When nuclei were selected for
anaphase I spindles (B), virtually all showed absent Cdc14-13Myc staining of
the DNA, as expected. Arrows indicate Cdc14-13Myc staining in the merged
images. Bars: 2 µm.

Supplementary Figure S10. Surface-spread nuclei stained for Zip1 and Smt3
simultaneously. Individual channels obtained for the merged images shown in
Figure 6G and H. Bars: 2 µm. Strains: wild type (Y940), ipl1-mn (Y1206).

Supplementary Figure S11. Red1 accumulation on spindles. (A)
Accumulation of Red1 at the poles of metaphase I spindles in wild type (~ 1/3,
Y940) and on anaphase I spindles in ipl1-mn (~ 1/3, Y1206). Bars: 2 µm.

Supplementary Figure S12. Hop1 dissociation from meiotic chromosomes in
wild type and ipl1-mn. Examples of Hop1 staining in nuclei at various stages
of meiosis I in wild type (Y940) and *ipl1-mn* (Y1206) (A and D). tub = tubulin. Bars: 2 µm. Quantification of Hop1 staining of meiotic chromosomes and aggregate formation in wild type (B and C) and *ipl1-mn* (E and F). > 50 nuclei were assessed for each stage.

**Supplementary Figure S13.** Rec8 is retained in anaphase I/telophase I nuclei of *ipl1-mn* that contain Zip1 staining. (A) Examples of wild-type (Y1485) nuclei stained for DNA (DAPI), Zip1 (red), tubulin (green), and Rec8-3HA (Rec8, blue). 30/30 spreads with clearly separated nuclei showed Rec8 staining at the spindle poles only. In contrast, 30/30 spreads that contained Zip1 staining in the *ipl1-mn* mutant (Y1551) also displayed significant non-polar Rec8 staining (B). Bars: 2 µm.

**Supplementary Figure S14.** Decoupling of cell cycle progression and SC disassembly in the *ipl1-mn*, but not the histone H3 Ser10→Ala mutant. (A) *ipl1-mn* (Y1175, S288c) shows delayed SC disassembly at diplotene, metaphase I and anaphase I. (B) SC disassembly occurs normally a mutant (and isogenic wild-type strain, Y1127) expressing histone H3 Ser10→Ala (Y1728, S288c). Zip1 is shown in green and tubulin (tub) in magenta. Bars: 2 µm.
Supplementary Tables.

**Supplementary Table S1: Strains used in this study.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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</table>
| Y940   | MATa _his4::URA3-arg4-EcPal(1691) LEU2_  
MATα _HIS4_ leu2::URA3-ARG4_  
ura3Δ  arg4Δ lys2 ho::LYS2  
ura3Δ  arg4Δ lys2 ho::LYS2 | (Allers and Lichten, 2001) |
| Y1175  | MATα _leu2-3,112 his3Δ lys2ΔBglII_  
MATa _leu2-3,112 his3Δ lys2ΔBglII_  
arg4 _ivl-Kpn, PAC2::[pD174::LEU2 lacO array]_  
arg4 _ivl-Kpn, PAC2::[pD174::LEU2 lacO array]_  
rad3 trp2  KANMX6-P_{CLB2}-3HA-IPL1  
rad3 trp2  KANMX6-P_{CLB2}-3HA-IPL1 | This work |
| Y1206  | MATa _his4::URA3-arg4-EcPal(1691) LEU2_  
MATα _HIS4_ leu2::URA3-ARG4_  
ura3Δ  arg4Δ lys2 ho::LYS2  
ura3Δ  arg4Δ lys2 ho::LYS2  
KANMX6-P_{CLB2}-3HA-IPL1  
KANMX6-P_{CLB2}-3HA-IPL1 | This work |
| Y1381  | MATα _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
MATa _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
ho::LYS2  
ho::LYS2 | This work |
| Y1485  | MATα _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
MATa _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
ho::LYS2  
PDS1-18MYC::TRP1  
ho::LYS2  
PDS1-18MYC::TRP1  
REC8-3HA::URA3  
REC8-3HA::URA3 | (Clyne et al., 2003) |
| Y1486  | MATα _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
MATa _leu2::hisG his3::hisG trp1::hisG ura3 lys2_  
ho::LYS2  
PDS1-18MYC::TRP1  
ho::LYS2  
PDS1-18MYC::TRP1  
REC8-3HA::URA3  
REC8-3HA::URA3 | (Clyne et al., 2003) |
| Y1530 | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 zip1△ | This work |
|-------|-------------------------------------------------|-----------|
| Y1538 | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | P_{GAL1}^+NDT80::TRP1 KANMX6-P_{CLB2}^−3HA-IPL1 |
|       | P_{GAL1}^+NDT80::TRP1 KANMX6-P_{CLB2}^−3HA-IPL1 |
| Y1551 | MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2  
ho::LYS2 PDS1-18MYC::TRP1 | This work |
|       | MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2  
ho::LYS2 PDS1-18MYC::TRP1 | This work |
|       | REC8-3HA::URA3 KANMX6-P_{CLB2}^−3HA-IPL1 |
|       | REC8-3HA::URA3 KANMX6-P_{CLB2}^−3HA-IPL1 |
| Y1553 | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | P_{GAL1}^+NDT80::TRP1 pdr5△::TRP1 |
|       | P_{GAL1}^+NDT80::TRP1 pdr5△::TRP1 |
| Y1581 | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3 | This work |
|       | P_{GAL1}^+NDT80::TRP1 CLB3-13MYC::TRP1 |
|       | P_{GAL1}^+NDT80::TRP1 CLB3-13MYC::TRP1 |
| Y1582 | MATα leu2::hisG his3::hisG trp1::hisG lys2 | This work |
| Y1583          | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | P_GAL1^-NDT80::TRP1  
|               | P_GAL1^-NDT80::TRP1  
|               | ipl1-M181G, T244A::LEU2 (ipl1-as5) 
|               | ipl1-M181G, T244A::LEU2 (ipl1-as5)  
|               | This work                           |
| Y1602          | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | P_GAL1^-NDT80::TRP1  
|               | P_GAL1^-NDT80::TRP1  
|               | (Carlile and Amon, 2008)             |
| Y1627          | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | P_GAL1^-NDT80::TRP1  
|               | P_GAL1^-NDT80::TRP1  
|               | KANMX6-P_CLB2^-3HA-CDC20  
|               | KANMX6-P_CLB2^-3HA-CDC20  
|               | This work                           |
| Y1656          | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | MATα leu2::hisG his3::hisG trp1::hisG lys2  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
|               | This work                           |
| Y1657 | $P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{KANMX6}-\text{P}_{\text{CLB2}}^{-}\text{3HA-CDC20}$ | This work |
| Y1658 | $\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$   
$\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{KANMX6}-\text{P}_{\text{SCC1}}^{-}\text{3HA-CDC5}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{KANMX6}-\text{P}_{\text{SCC1}}^{-}\text{3HA-CDC5}$ | This work |
| Y1661 | $\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$   
$\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$  
$\text{ho::LYS2} \; \text{zip1}^{\Delta} \; \text{KANMX6}-\text{P}_{\text{CLB2}}^{-}\text{3HA-IPL1}$  
$\text{ho::LYS2} \; \text{zip1}^{\Delta} \; \text{KANMX6}-\text{P}_{\text{CLB2}}^{-}\text{3HA-IPL1}$ | This work |
| Y1662 | $\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$   
$\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{CLB1-13MYC::TRP1}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{CLB1-13MYC::TRP1}$ | This work |
| Y1663 | $\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$   
$\text{MAT}^{\alpha} \; \text{leu2::hisG} \; \text{his3::hisG} \; \text{trp1::hisG} \; \text{lys2}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$\text{ho::LYS2} \; \text{ara3::pGPD1-GAL4}(848).\text{ER}::\text{URA3}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{CLB1-13MYC::TRP1}$  
$P_{\text{GAL1}}^{-}\text{NDT80::TRP1} \; \text{CLB1-13MYC::TRP1} \; \text{KANMX6}-\text{P}_{\text{CLB2}}^{-}\text{3HA-IPL1}$  
$\text{KANMX6}-\text{P}_{\text{CLB2}}^{-}\text{3HA-IPL1}$ | This work |
<p>| Y1664 | $\text{MAT}^{\alpha} ; \text{leu2::hisG} ; \text{his3::hisG} ; \text{trp1::hisG} ; \text{lys2}$ | This work |</p>
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<td>Y1669</td>
<td>MATα leu2::hisG his3::hisG trp1::hisG lys2, ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3, ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3, P&lt;sub&gt;GAL1&lt;/sub&gt;-NDT80::TRP1 CDC14-13MYC::TRP1, P&lt;sub&gt;GAL1&lt;/sub&gt;-NDT80::TRP1 CDC14-13MYC::TRP1, KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1, KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1</td>
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<td>MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2, MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2, ho::LYS2 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1, ho::LYS2 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1</td>
<td>(Liu et al., 2005)</td>
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<td>Y1728</td>
<td>MATα leu2 hht1-hhf1Δ::KAN hhf2-hht2Δ::NAT, MATα leu2 hht1-hhf1Δ::KAN hhf2-hht2Δ::NAT, hta1-htb1Δ::HPH hta2-htb2Δ::NAT, hta1-htb1Δ::HPH hta2-htb2Δ::NAT, p(HTA1-HTB1-HHT2-HHF2)-LEU2</td>
<td>(Liu et al., 2005)</td>
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<td>Y2030</td>
<td>MATα leu2::hisG his3::hisG trp1::hisG lys2, MATα leu2::hisG his3::hisG trp1::hisG lys2, ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3, ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3, P&lt;sub&gt;GAL1&lt;/sub&gt;-NDT80::TRP1 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1, P&lt;sub&gt;GAL1&lt;/sub&gt;-NDT80::TRP1 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1, KANMX6-P&lt;sub&gt;SCC1&lt;/sub&gt;-3HA-CDC5, KANMX6-P&lt;sub&gt;SCC1&lt;/sub&gt;-3HA-CDC5</td>
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<td>Y2262</td>
<td>MATα leu2::hisG his3::hisG trp1::hisG lys2, MATα leu2::hisG his3::hisG trp1::hisG lys2</td>
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*Strains were SK1 or, when indicated, S288c.

**Supplementary References.**

