Supplemental data

**Supplemental Table S1.** Yeast strains used in this study. Strains are from the W303-1a background (*ade2-1 trp1-1 ura3-1 leu2-3,112 his3-11,15 can1-100 RAD5*) except strains RM11-1a, 234-1a and 234-1d (RM11 background) and strain AC021 (S288c background).

| Strain     | Genotype                                                                 |
|------------|---------------------------------------------------------------------------|
| Lev1212    | $MAT\alpha\ bar1-\Delta\ pACE1-\text{UBR1}\ pACE1-\text{ROX1}\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 (Pobiega and Marcand 2010) |
| Lev752     | $MAT\alpha\ bar1-\Delta\ KANr-pGAL1-CEN6-pGAL1$-skHIS3                    |
| Lev801     | $MAT\alpha\ bar1-\Delta\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-7L       |
| Lev802     | $MAT\alpha\ bar1-\Delta\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-7L       |
| Lev822     | $MAT\alpha\ bar1-\Delta\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-Y'-14     |
| Lev826     | $MAT\alpha\ bar1-\Delta\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-Y'-5      |
| RM11-1a    | $MAT\alpha\ ho::loxP-KANr-loxP\ leu2-DO\ ura3-DO$                         |
| 234-1a     | $MAT\alpha\ ho::loxP-KANr-loxP\ leu2-DO\ ura3-DO\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 |
| 234-1d     | $MAT\alpha\ ho::loxP-KANr-loxP\ leu2-DO\ ura3-DO\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-7L |
| 234-1a/dic | $MAT\alpha\ ho::loxP-KANr-loxP\ leu2-DO\ ura3-DO\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 |
| 234-1d/dic | $MAT\alpha\ ho::loxP-KANr-loxP\ leu2-DO\ ura3-DO\ KANr-pGAL1-CEN6-pGAL1$-skHIS3 6R-klLEU2-7L |
| Lev1258    | Lev728 GalR clone n°V complemented with pBP43 (RAP1) (Pobiega and Marcand 2010) |
Lev1258/TRP1  Lev1258 6R-X-klTRP1-Y'-7R

Lvl83  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1

Lvl103  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1 6R-klLEU2-7L

Ynb15  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1  chro 6_coord._97078::CEN4-klLEU2

Lev803  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 6R-klLEU2-7L

Lvl200  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 rap1-(Δ)::KANr
cdc15-2 GalR clone n°I complemented with pBP43 (RAP1) 6+14 Tel-Tel

Lev1276  Lev728 GalR clone n°VIII complemented with pBP43 (RAP1) (Pobiega and Marcand 2010)

Lvl201  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 rap1-(Δ)::KANr GalR
clon n°I complemented with pBP43 (RAP1) and sp534 (CDC15) 6+14 Tel-Tel

Lvl242  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 GFP-TUB1::URA3 MYO1-yeGFP::HPH 6R-klLEU2-7L

Lvl119  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 chro 6_coord._97078::CEN4-klLEU2

Lvl198  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 rap1-(Δ)::KANr
cdc15-2 GalR clone n°I complemented with pBP43 (RAP1) 6+3 Tel-Tel

Lvl237  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR19myc::URA3 MYO1AtD-9myc::hphB 6R-klLEU2-7L

Lvl238  MATα bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR19myc::URA3 6R-klLEU2-7L
| Level | Organism | Strain Description |
|-------|-----------|--------------------|
| Lvl233 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-<br>AtTIR1<sup>9myc</sup>::URA3 chro 6_coord._97078::CEN4-klLEU2 | |
| Lvl236 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-<br>AtTIR1<sup>9myc</sup>::URA3 MYO1<sup>16D-9myc</sup>::hphB chro 6_coord._97078::CEN4-klLEU2 | |
| Lvl246 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-<br>AtTIR1<sup>9myc</sup>::URA3 MYO1<sup>16D-9myc</sup>::hphB 6R-telomere fusion-klLEU2-7L | |
| Lvl252 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-<br>AtTIR1<sup>9myc</sup>::URA3 6R- telomere fusion-klLEU2-7L | |
| AC021 | MATa lys2-801 his3Δ200 leu2-3,112 GFP-TUB1::URA3 | |
| Lev819 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 | |
| Lev819/dic | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 6R-klLEU2-7L | |
| Lev816 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 GFP-TUB1::URA3 | |
| Lev816/dic | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 GFP-TUB1::URA3 6R-klLEU2-7L | |
| Lev856 | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 SPC42-mCherry::HPH lacI**-GFP::URA3 chro 7_coord_484500::lacO_array::TRP1 | |
| Lev856/dic | MATa bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 SPC42-mCherry::HPH lacI**-GFP::URA3 chro 7_coord_484500::lacO_array::TRP1 6R-klLEU2-7L | |
Supplemental Figure S1. Survival to a 6+7 dicentric. (A) Overnight cultures of strains Lev346 (WT), Lev752 (conditional monocentric), Lev801 and Lev802 (two independent clones with a conditional 6+7 dicentric), Lev838/dic1 and Lev838/dic2 (two independent clones with a conditional 6+7 dicentric in Lig4 deficient cells) and Lev838 (lig4-Δ) were diluted by 10-fold serial dilutions, plate on galactose synthetic medium and on glucose rich medium, and incubated for 3 d (galactose) or 2 d (glucose) at 30°C (left panels; the upper part is shown of Figure 1C). Cells with a dicentric generate slow growing colonies on glucose. A few normally growing colonies appear among the lower dilution of the LIG4+ cells. Then, colonies from the third dilution on glucose (circled) were scratched from the plate, grown overnight in rich medium and spotted again on galactose synthetic medium and on glucose rich medium (right panels). Cells previously containing a dicentric generate normally growing colonies on glucose. This indicates that some cells in the slow growing colonies adapted to the presence of a dicentric and restored a normal growth. From LIG4+ cells, a large fraction of these adapted cells are resistant to galactose, suggesting a loss of the conditional centromere. From lig4-Δ cells, most are sensitive to galactose, suggesting a conservation of the conditional centromere. (B) Independent slow growing colonies on glucose were streaked on glucose to isolate independent normally growing adapted cells. Among the 48 survivors isolated from LIG4+ cells, 35 (72%) were resistant to galactose. Among the 42 survivors isolated from lig4-Δ cells, 2 (5%) were resistant to galactose. As expected, these galactose-resistant clones have lost CEN6 (as determined by PCR) and display an intact 6+7 fused chromosome on PFGE (e.g. lanes 3-6). The galactose sensitive clones have conserved the conditional CEN6, as expected. PFGE and Southern blotting with probes from the distal arms of the original dicentric shows that each Gal S clone fragmented the dicentric into two distinct chromosomes of various sizes. The sums of the two chromosomes are larger than the original 6+7 dicentric, indicating that broken end stabilization was associated with a gain of sequence, presumably through break-induced replication with other chromosomes (e.g. lanes 7-10 in LIG4+ cells, lanes 13-14 and 16-18 in lig4-Δ cells; lane 15: the missing fragment may be a circular chromosome with one of the centromere). Survivors with an intact 6+7 fused chromosome and a deletion of CEN7 were not isolated. This may be due to the presence of an essential gene immediately adjacent to CEN7. The palindrome created by the pGAL1 insertions at CEN6 may also facilitate the lost of CEN6 prior ligation to the other end, perhaps explaining that survivors with a
CEN6 deletion predominate in this assay. The latter hypothesis is supported by a similar bias in favour of CEN6 lost observed with 6+5 and 6+14 dicentrics as well as with a dicentric created by CEN4 insertion in chromosome 6 right arm (data not shown).

**Supplemental Figure S2.** Dicentrics with 30 and 45 kb intercentromeric distance do not break in anaphase. The CEN4-klLEU2 cassette was inserted in chromosome 6 right arm at 30 or 45 kb from CEN6 (coordinate 176900 and 193800 respectively) of strain 161-16b to create strains Lvl123 and Lvl128 respectively. Cells from these two strains were grown exponentially in galactose-containing medium at 25°C, synchronized in G1, released in glucose-containing medium and either blocked in G2/M at 25°C, allowed to proceed to next G1 at 25°C and blocked in anaphase at 36°C. Cells arrested at 36°C were released at 25°C. Chromosomes were separated by PFGE and probed with a fragment from chromosome 6 left arm.

**Supplemental Figure S3.** Chromosomes were separated by PFGE and labelled with Gel Red prior blotting. These gels correspond to the Southern blots shown in Figure 3, 4 & 5.
Supplemental Figure S1

A

WT
conditional monocentric
conditional 6+7 dicentric
conditional 6+7 dicentric *lig4*Δ
conditional monocentric *lig4*Δ

B

|       | *LIG4* survivors to glucose | *lig4*Δ survivors to glucose |
|-------|----------------------------|-----------------------------|
|       | Gal R | Gal S | no dicentric | 6-7 dicentric | Gal R | Gal S | no dicentric | 6-7 dicentric |
|       |       |       |             |              |       |       |             |              |
|       |       |       |             |              |       |       |             |              |
|       |       |       |             |              |       |       |             |              |

Galactose resistant clones:

*cen6*-Δ

Galactose sensitive clones:

*CEN6*
Supplemental Figure S2

CEN4 at 30 kb from CEN6

CEN4 at 45 kb from CEN6

G2/M @ 25°C
next G1 @ 36°C
released @ 25°C

G2/M @ 25°C
next G1 @ 36°C
released @ 25°C

CEN4

CEN6
Supplemental Figure S3