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**a** bar1-Δ pACE1-UBR1 pACE1-ROX1 KANr-pGAL1-CEN6-pGAL1-skHIS3* (Pobiega and Marcand 2010)
Lev752	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3*
Lev801	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 6R-klLEU2-7L*
Lev802	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 6R-klLEU2-7L*
Lev822	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 6R-klLEU2-Y'-14*
Lev826	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 6R-klLEU2-Y'-5*
RM11-1a	*MAT**a** ho::loxP-KANr-loxP leu2-DO ura3-DO*
234-1a	*MAT**a** ho::loxP-KANr-loxP leu2-DO ura3-DO KANr-pGAL1-CEN6-pGAL1-skHIS3*
234-1d	*MAT**a** ho::loxP-KANr-loxP leu2-DO ura3-DO KANr-pGAL1-CEN6-pGAL1-skHIS3 6R-klLEU2-7L*
234-1a/dic	*MAT**a** ho::loxP-KANr-loxP leu2-DO ura3-DO KANr-pGAL1-CEN6-pGAL1-skHIS3*
234-1d/dic	*MAT**a** 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	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1*
Lvl103	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1 6R-klLEU2-7L*
Ynb15	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 sae2-Δ::NATr exo1-Δ::klTRP1 chro 6_coord._97078::CEN4-klLEU2*
Lev803	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 6R-klLEU2-7L*
Lvl200	*MAT**a** 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	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 rap1-(Δ)::KANr* GalR clone n°I complemented with pBP43 (*RAP1*) and sp534 (*CDC15*) *6+14 Tel-Tel*
Lvl242	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 GFP-TUB1::URA3 MYO1-yeGFP::HPH 6R-klLEU2-7L*
Lvl119	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 cdc15-2 chro 6_coord._97078::CEN4-klLEU2*
Lvl198	*MAT**a** 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	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 MYO1$^{AID-9myc}$::hphB 6R-klLEU2-7L*
Lvl238	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 6R-klLEU2-7L*

Lvl233	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 chro 6_coord._97078::CEN4-klLEU2*
Lvl236	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 MYO1$^{AID-9myc}$::hphB chro 6_coord._97078::CEN4-klLEU2*
Lvl246	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 MYO1$^{AID-9myc}$::hphB 6R-telomere fusion-klLEU2-7L*
Lvl252	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADH1-AtTIR1^{9myc}::URA3 6R- telomere fusion-klLEU2-7L*
AC021	*MATα lys2-801 his3Δ200 leu2-3,112 GFP-TUB1::URA3*
Lev819	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1*
Lev819/dic	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 6R-klLEU2-7L*
Lev816	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 GFP-TUB1::URA3*
Lev816/dic	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 MYO1-yeGFP::HPH SPC42-yeGFP::klTRP1 GFP-TUB1::URA3 6R-klLEU2-7L*
Lev856	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 SPC42-mCherry::HPH lacI**-GFP::URA3 chro 7_coord._484500::lacO_array::TRP1*
Lev856/dic	*MAT**a** bar1-Δ KANr-pGAL1-CEN6-pGAL1-skHIS3 ADE2 SPC42-mCherry::HPH lacI**-GFP::URA3 chro 7_coord._484500::lacO_array::TRP1 6R-klLEU2-7L*

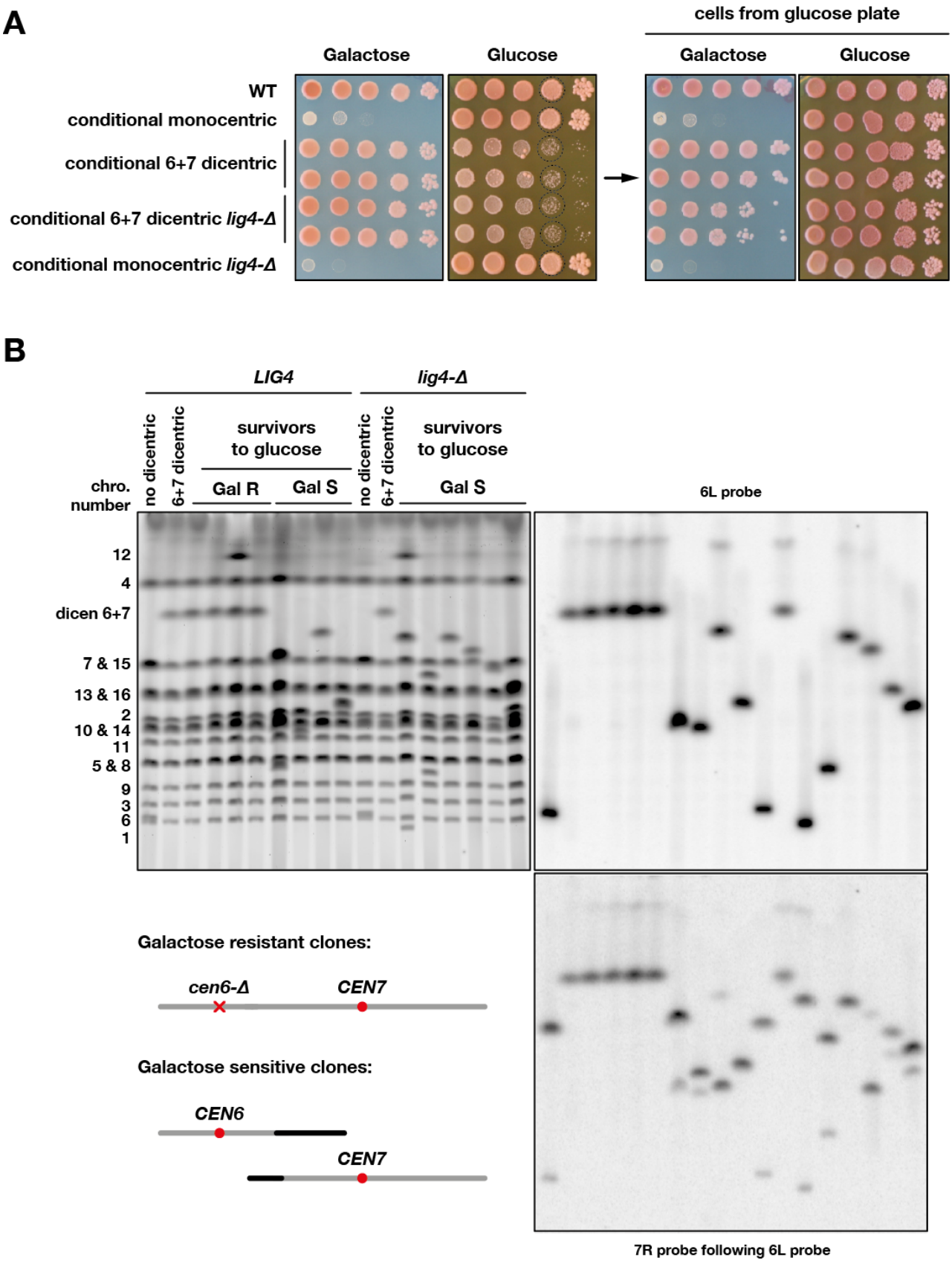
Supplemental Figure S1. Survival to a 6+7 dicentric. (A) Overnight cultures of strains Lev346 (WT), Lev752 (conditional monocentric), Lev801 and Lev802 (two independent clones with a conditional 6+7 dicentric), Lev838/dic1 and Lev838/dic2 (two independent clones with a conditional 6+7 dicentric in Lig4 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).

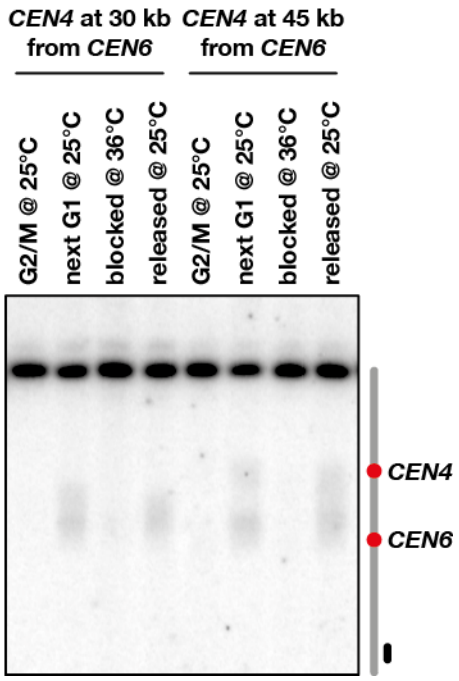
Supplemental Figure S2. Dicentrics with 30 and 45 kb intercentromeric distance do not break in anaphase. The *CEN4-klLEU2* cassette was inserted in chromosome 6 right arm at 30 or 45 kb from *CEN6* (coordinate 176900 and 193800 respectively) of strain 161-16b to create strains Lvl123 and LvL128 respectively. 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



Supplemental Figure S2



Supplemental Figure S3