

Supporting Information

Nikitin et al. 10.1073/pnas.0800464105

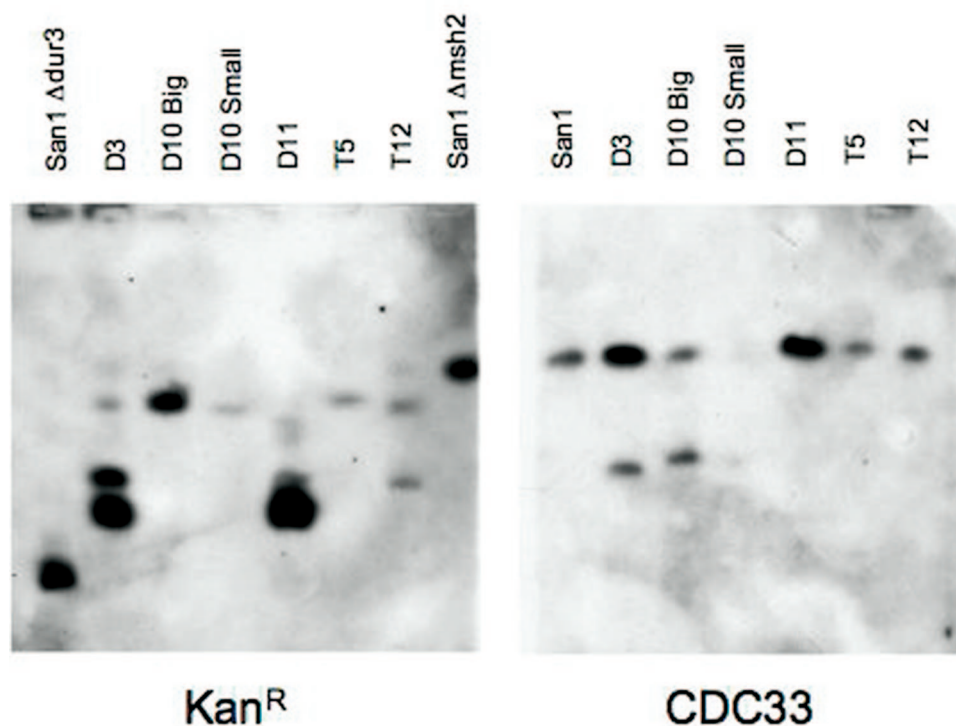


Fig. S1. Southern analysis of various yeast strains with Kan^R (Left) and CDC33 (Right) probes, respectively. (Left) Strains: San1 (*DUR3::KAN^R*), D3, D10 Big, D10 Small, D11, T5, T12, San1 (*MSH2::KAN^R*) [Tosato V, Waghmare SK, Bruschi CV (2005) Non-reciprocal chromosomal bridge-induced translocation (BIT) by targeted DNA integration in yeast. *Chromosoma* 114:15–27]. (Right) Strains: San1, D3, D10 Big, D10 Small, D11, T5, T12. Chromosomal bands of strains T5 and T12 were not considered for the calculation of the best-fitting curve of DNA migration because they are identical to those of strains D3 and D11. An overexposure of the film was used to visualize D10 Small bands better.

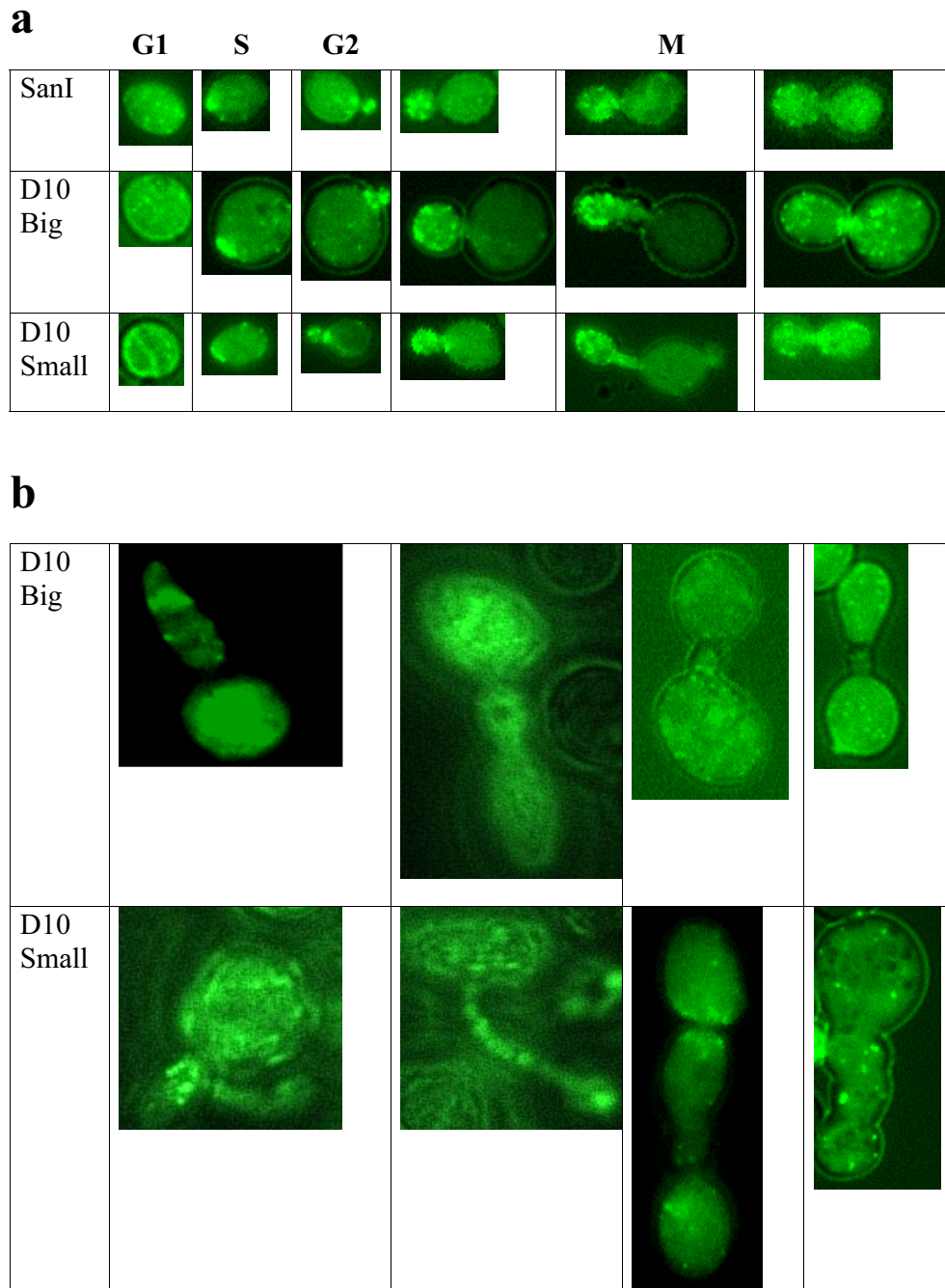


Fig. S2. Fluorescence microphotography of FITC–phalloidin-stained translocant cells to reveal actin distribution. Panels are of different size to reproduce all cells at similar size for better comparison. (a) SanI and D10 translocants at different stages of the cell cycle. (b) Abnormal actin distribution in D10 Big and Small translocants in G₂ and M phases of the cell cycle.

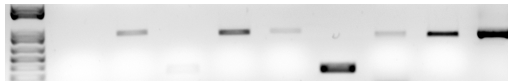
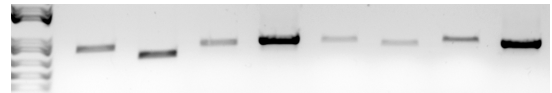
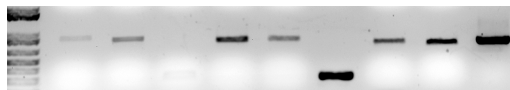
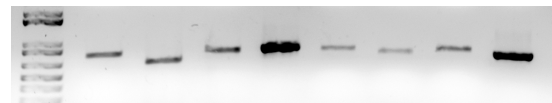
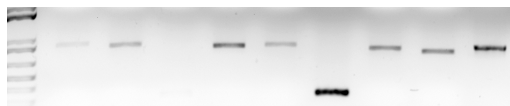
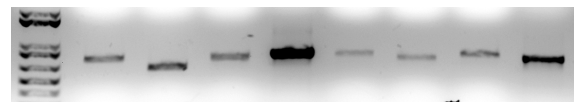
San I – VIII chromosome**San I – XV chromosome****D10 Big – VIII chromosome****D10 Big – XV chromosome****D10 Small – VIII chromosome****D10 Small – XV chromosome**

Fig. S3. Quantitative RT-PCR bands patterns representing the expression of the genes reported in the same order in Fig. 3.

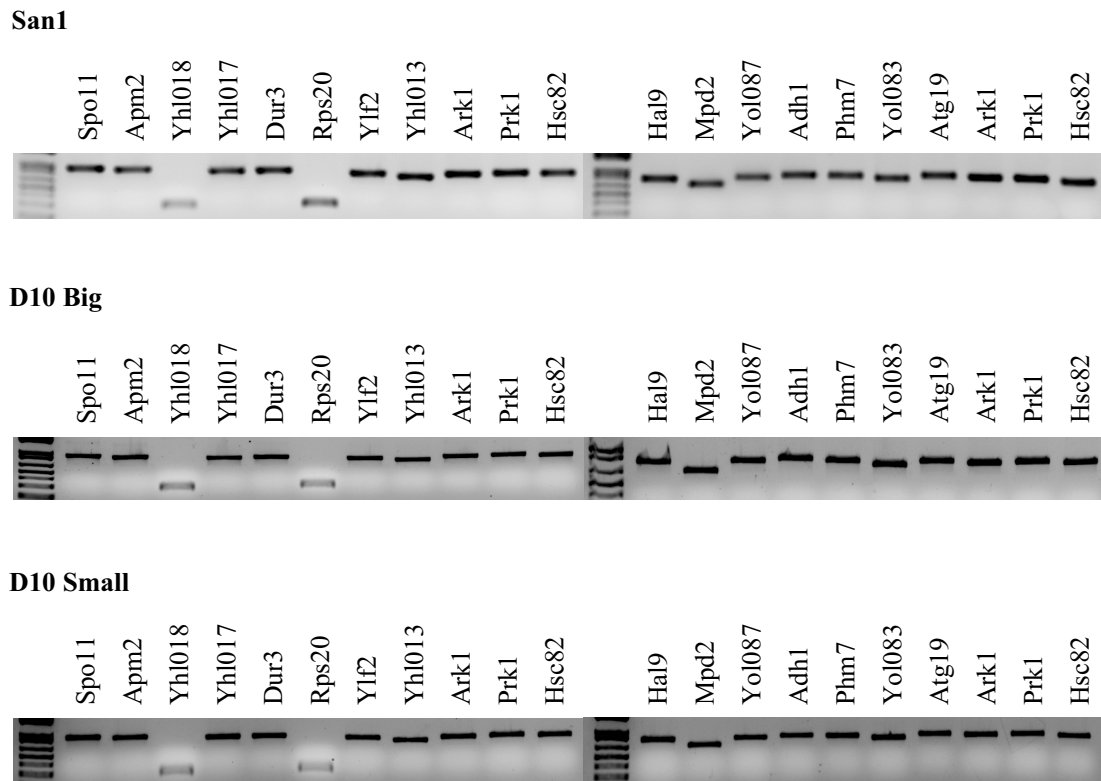
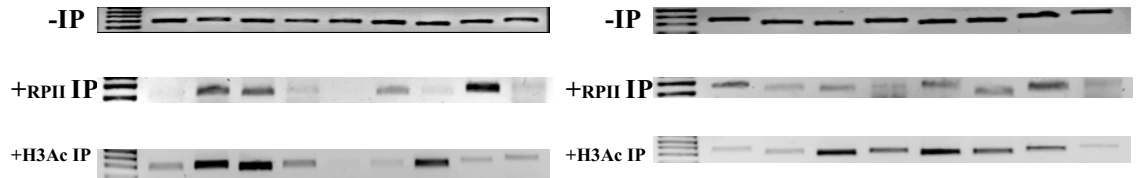


Fig. S4. Quantitative PCR copy number control of DNA amount at the translocation breakpoints. Using the same primer sets of RT-PCR analysis of genes expression, we compared DNA amount at translocation breakpoints with that of genes on other chromosomes (chromosome IX, *PRK1*; chromosome XIV, *ARK1*; and chromosome XIII, *HSC82*). No differences were observed in both parental San1 and D10 translocant strains.

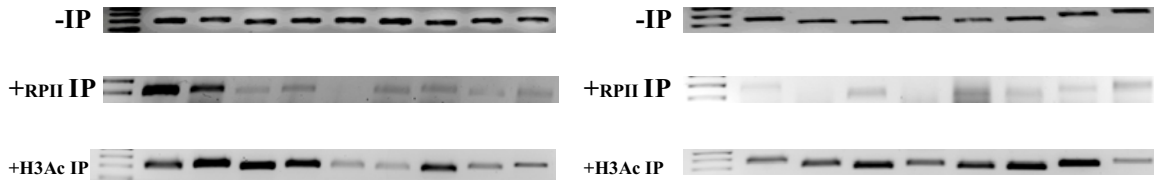
San I – VIII chromosome

San I – XV chromosome



D10 Big – VIII chromosome

D10 Big – XV chromosome



D10 Small – VIII chromosome

D10 Small – XV chromosome

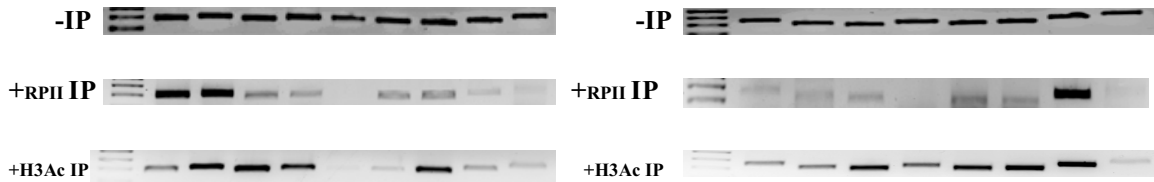


Fig. S6. Continued

Table S1. Names, functions, and locations of genes used for RT-PCR and ChIP experiments.

Genes located at translocation breakpoint on VIII chromosome

SPO11—Meiosis-specific protein that initiates meiotic recombination by catalyzing the formation of double-stranded breaks in DNA via a transesterification reaction, 7.87 kb from *DUR3*.
APM2—Protein of unknown function, homologous to the medium chain of mammalian clathrin-associated protein complex; involved in vesicular transport, 4.3 kb from *DUR3*.
YHL018W—Putative protein of unknown function; GFP fusion protein localizes to mitochondria, 1.96 kb from *DUR3*.
YHL017W—Putative protein of unknown function; GFP fusion protein colocalizes with clathrin-coated vesicles, 0.16 kb from *DUR3*.
DUR3—Plasma membrane urea transporter.
RPS20—Protein component of the small (40S) ribosomal subunit, 1.17 kb from *DUR3*.
YLF2—Protein of unknown function; shares weak similarity to *Escherichia coli* GTP-binding protein gtp1, 1.85 kb from *DUR3*.
YHL013C, OTU2—Putative protein of unknown function, member of the ovarian tumor-like (OTU) superfamily of predicted cysteine proteases, 3.19 kb from *DUR3*.

Genes located at translocation breakpoint on XV chromosome

HAL9—Putative transcription factor containing a zinc finger, 6 kb from *ADH1*.
MPD2—Member of the protein disulfide isomerase (PDI) family; exhibits chaperone activity, 4.8 kb from *ADH1*.
YOL087C—Hypothetical protein, 0.9 kb from *ADH*.
ADH1—Alcohol dehydrogenase.
PHM7—Protein of unknown function; expression is regulated by phosphate levels; GFP fusion protein localizes to the cell periphery and vacuole, 1.76 kb from *ADH1*.
YOL083W—Hypothetical protein, 5.12 kb from *ADH1*.
ATG19—Protein involved in the cytoplasm-to-vacuole targeting pathway and in autophagy, recognizes cargo proteins and delivers them to the preautophagosomal structure for eventual engulfment by the autophagosome and degradation, 8.1 kb from *ADH1*.

Genes located far from translocation breakpoints

CBP2—Codes for mitochondrial splicing protein, VIII chromosome left arm subtelomeric region, ≈50 kb from translocation breakpoint to the left.
PPE1—Codes for small subunit mitochondrial ribosomal protein, VIII chromosome, ≈170 Kb from translocation breakpoint to the right.
RRP40—Codes for rRNA-processing protein, XV chromosome; ≈100 kb from translocation breakpoint to the left.
TOP1—Codes for topoisomerase I, XV chromosome, ≈150 kb from translocation breakpoint to the right.
CYT1—Codes for cytochrome C1, XV chromosome, ≈300 kb from translocation breakpoint to the right.

Control gene

HSC82—Cytoplasmic chaperone of the Hsp90 family, redundant in function and nearly identical with Hsp82p, and together they are essential; expressed constitutively at 10-fold higher basal levels than Hsp82 and induced 2- to 3-fold by heat shock.

Table S2. Primers used in this work (from the 5' end to the 3' end). The size of the amplicons is in base pairs.

Primers used for production of BIT translocants

D3, D10, D11 translocants

Adh1 For-63: CTTTCTTCCTTGTTCCTTTCTGCACAATATTTCAAGCTATACCAAGCATACAATCAACTATCCGCGCGTTGGCCGATTCAT
Dur3 P-65: TACCAGCGCCTTGAGGTAGCGGAGGTTAAATTTCTCCATACTATAACAATGACAAATTTATGTGTCGACGGATCCCCGGGTAA

AD5 translocant

Ald5 For-65: GCTCAAAGAACAGAACAAAACACGATTATATAAGCCCATGTAAAAAGAAGCTCTTAATTTATTTTCGCGCGTTGGCCGATTCAT
Dur3 P-65: TACCAGCGCCTTGAGGTAGCGGAGGTTAAATTTCTCCATACTATAACAATGACAAATTTATGTGTCGACGGATCCCCGGGTAA

SUSU1 and SUSU2 translocants

SSU1 For-65:
TCTCAGTATATTTTGTGCTTTCCTTCATATGTATATATATCTATTTACATATTAGTTTACAGAACGCGCGTTGGCCGATTCAT
SUC2 Rev-65:
CTTTGCTGGGGGAGCGAGAAGCTACGCTAGGACAACAACCTCCCATACGGTAAATGTCTTAGTATTGACGGATCCCCGGGTAA

Primers used for RT-PCR and PCR for copy number

Spo11 For: ATACAAGCAGTCTGACATTCACAC
Spo11 Rev: GTTCATTTAGCTTTCTTTGGAAA
Size of amplicon: 928

Apm2 For: ATGCCCAAAGTGAAGAACAAAGATCCATAA
Apm2 Rev: TATATGCGTACTCTTCGTCGCTGAC
Size of amplicon: 885

YHL018W For: ATTGTTAGAATCGCGTCCAGTG
YHL018W Rev: GTCTATATCGCTAAGCTGGCTGTGA
Size of amplicon: 299

YHL017W For: TGGAAGCATAGACATGAGTTACTGC
YHL017W Rev: TATCCATATTGTGATCGTGTTCCTG
Size of amplicon: 837

Dur3 For: GCAGTATTACCTGTCGTTTTGACCT
Dur3 Rev: GATTGTCTATGTCTCTCGCACTAA
Size of amplicon: 856

Rps20 For: GGAAAAGTTGAAGAACAAGAACAA
Rps20 Rev: AGGTTCAATGGTGATTGAGTGATT
Size of amplicon: 312

Yif2 For: GCGTACCTGGAACTCACAATATA
Yif2 Rev: ATAAATGTTTCACGCAAGTCGCTAT
Size of amplicon: 819

YHL013C For: GAGACATCGCAAGGAAAATAAAGAT
YHL013C Rev: ATAGGTCTTCCACTCATCAGAATGC
Size of amplicon: 767

Hal9 For: TGATAACGAACGCCTATACAAAGGT
Hal9 Rev: TAGACCACTGGAAAGAGTATCTCCG
Size of amplicon: 810

Mpd2 For: ATTTTCCGTATTATCAACATGCGTC
Mpd2 Rev: CTTCTAGTCTTCGCTTTCTTTTC
Size of amplicon: 679

YOL087C For: CTTCCAGTCACCAAGATAATCAAGG
YOL087C Rev: CACCCTGAGATTTCCAGTAAAGTGT
Size of amplicon: 834

Adh1 For: CCATTGCCAGTTAAGCTACCATTAG
Adh1 Rev: TGTCACAACGTATCTACCAACGAT
Size of amplicon: 870

Phm7 For: GGCTATTCCTATCCTACATCGGACT
Phm7 Rev: TTAGCCTCAGGTTTATAATCAGGAG
Size of amplicon: 853

YOL083W For: AAGTAAGGCTCAAAACCAAGTGTG
YOL083W Rev: TTTCTTCCAAGTAAATGGCCTACT
Size of amplicon: 799

Atg19 For: CTCAGTTTATATCGGAGAAGGAACG
Atg19 Rev: TTTGAAAGGAGTAAATGGCTCTTG
Size of amplicon: 871

Hsc82 For: TACATGAGGACACTCAAAACAGAGC
Hsc82 Rev: TAATCAACTTCTTCCATCTCGGTGT
Size of amplicon: 843

Clb1 For: TAGAGCAGGATGACCAGAAAAAGTT
Clb1 Rev: TCGTCGTGAATAGTAGATCCAACAA
Size of amplicon: 804

CBP2 For: CCCTGGAGACATTTAAGAGCTACA
CBP2 Rev: CCTCGTTGCTTTTCTTCTCGTATAA
Size of amplicon: 887

PPE1 For: TCAAATACCACTTCAATCCCATCT
PPE1 Rev: TTTTGTGTTGACCCCAATTAGTCTT
Size of amplicon: 865

RRP40 For: GTCTACGTTTATATCCCTGGTGAT
RRP40 Rev: TAGATAATTCCTCGCACTTAACCCA
Size of amplicon: 605

TOP1 For: ATGAGCATGTTACTTTGAAACCTCC
TOP1 Rev: CCCATTTGAATTTTTCTTTAGGGT
Size of amplicon: 957

CYT1 For: AGTTTCTACTCTACCGCAACAGGTG
CYT1 Rev: ATCTTTTCTTTGTCATGTTTCAAGG
Size of amplicon: 757

ARK1 For: GAAAATACGATGCCGAACGCTAC
ARK1 Rev: GTTTTGGAGGAGGTTTAGGCTTTAA
Size of amplicon: 850

Prk1 For: AGCAAATCACTAAGCTCCACAAAAG
Prk1 Rev: AATCGGCAGTTTCTTTGATTGTAAA
Size of amplicons: 873

Primers used for ChIP

Spo11_prom_for: AAGAAGCCGTCAGTTTCTTTTGTG
Spo11_prom_rev: GGAGTGACCATTGGAGTTCAAGTGA
Size of amplicon: 305

Apm2_prom_for: CGAGTAGTTTGCCCTGTGAG
Apm2_prom_rev: TTTTTGAACTTGAAAGAACCAGC
Size of amplicon: 321

Yhl018_prom_for: TGGCTCCAGATTTTCATCCAGG
Yhl018_prom_rev: GGTCCCATTGAACTTCCAACGG
Size of amplicon: 301

Yhl017_prom_for: GGCAGGGCAGGTCTAAAAGGC
Yhl017_prom_rev: AATACATCCCTGAACAGACATCGTG
Size of amplicon: 314

Dur3.chip_for: ATGGGAGAATTTAAACCTCCGCTAC