

# **Supplementary Data**

**for**

## **Subtelomeres constitute a safeguard for gene expression and chromosome homeostasis**

Sanki Tashiro<sup>1</sup>, Yuki Nishihara<sup>1</sup>, Kazuto Kugou<sup>2</sup>, Kunihiro Ohta<sup>2</sup> & Junko Kanoh<sup>1,\*</sup>

<sup>1</sup>Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan

<sup>2</sup>Department of Life Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902,  
Japan

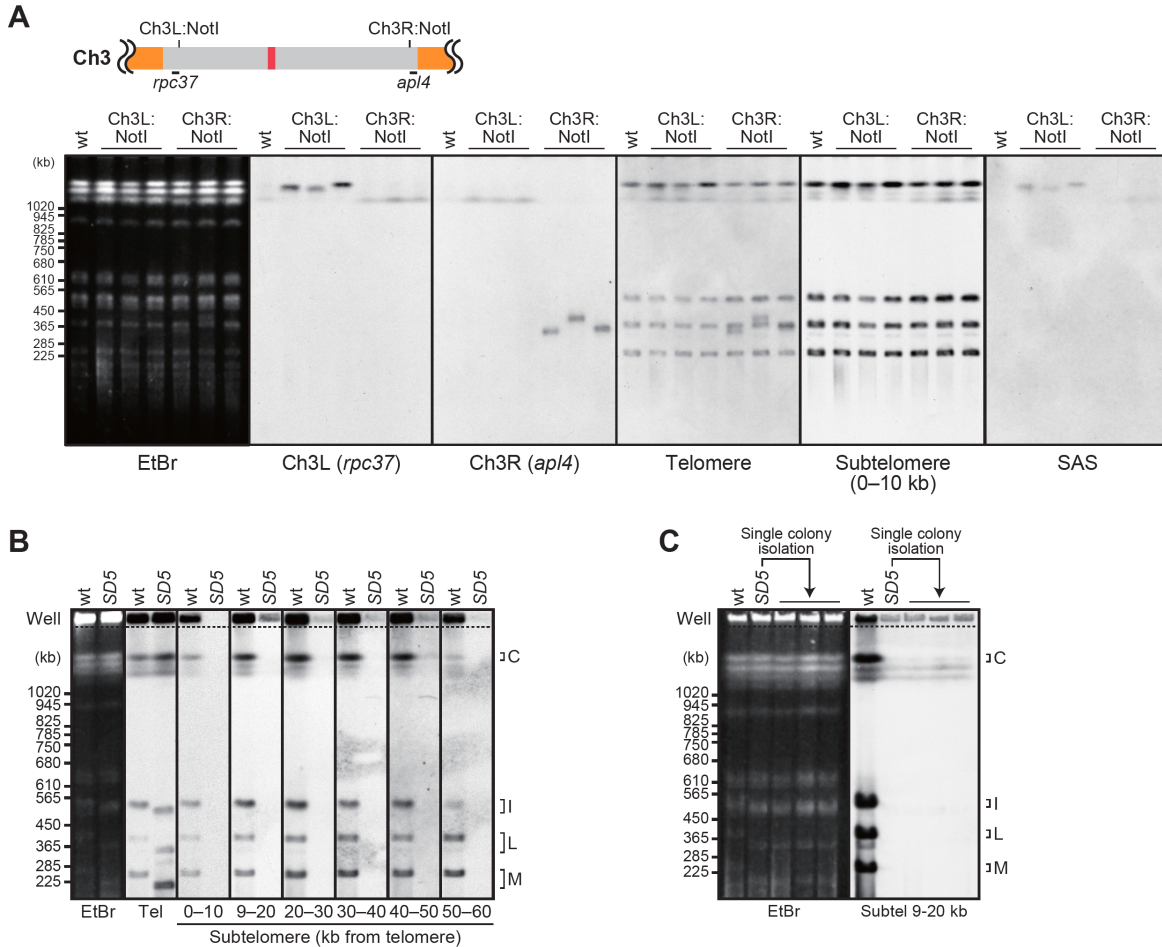
To whom correspondence should be addressed. Tel: +81 6 6879 4328; Fax: +81 6 6879  
4329; Email: jkanoh@protein.osaka-u.ac.jp

Present address: Kazuto Kugou, Department of Frontier Research, Kazusa DNA Research  
Institute, Kisarazu, Chiba 292-0818, Japan

Contents: Figures S1–S10

Tables S1–S4

## Supplementary Figures



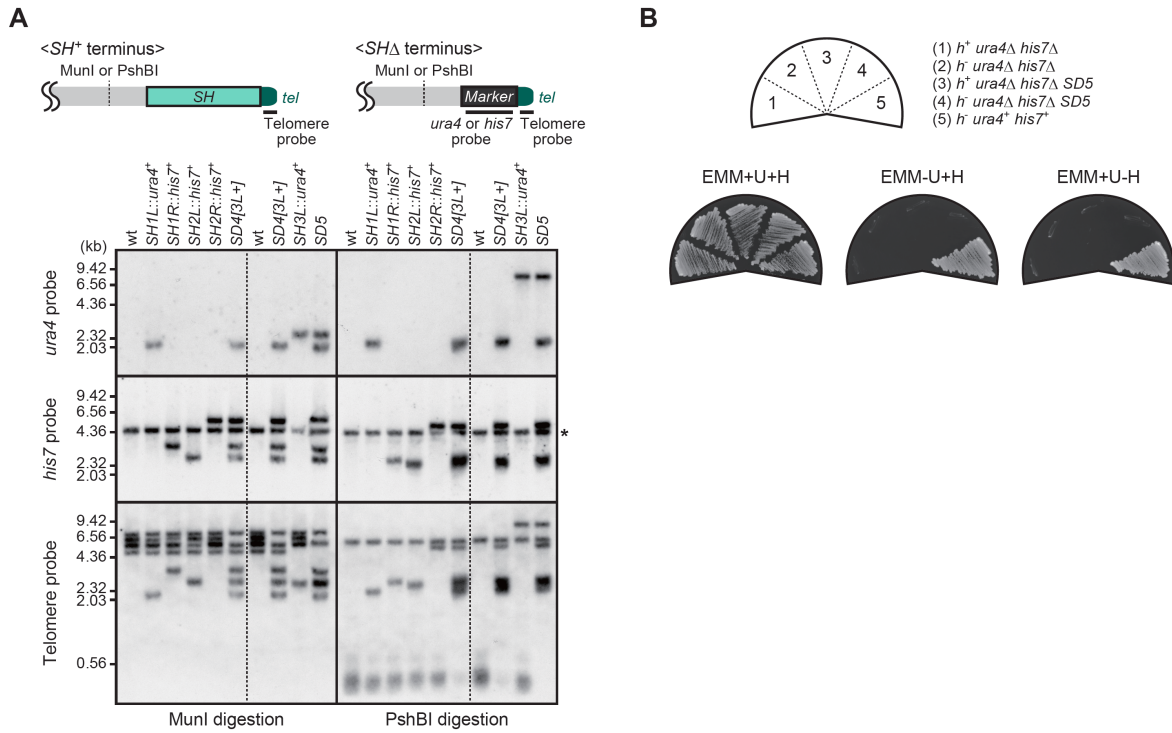
### Figure S1. Construction of the *SD5* strain

(A) Determination of the location of the *SH* regions in chromosome 3 of *S. pombe*. Sequences recognized by the *NotI* enzyme were introduced into the *rpc37* or and *apl4* loci of the left and right arms of chromosome 3, respectively (Ch3L:*NotI* and Ch3R:*NotI*, top panel), to enable the telomere-containing fragments of chromosome 3 to migrate different distances into gels during PFGE (note that the original chromosome 3 does not contain a *NotI* recognition site). Three independent clones of each insertion mutant were examined along with the parental wild-type strain. Genomic DNA was digested with *NotI* and analyzed by PFGE followed by Southern blotting. When the Southern blot membrane

was hybridized with probes specific for each arm, the signals from the left and right arms of chromosome 3 were detected at positions above the 1,020 kb marker and at approximately 400 kb, respectively (*rpc37* and *apl4*). When the membrane was hybridized with a probe for the SAS sequence, signals were detected only at the position of the left arm of chromosome 3, although the subtelomere (0–10 kb) signals were masked by those of the right arm of chromosome 2 (C fragments). Note that the differences in band size among the clones are probably due to copy number variation of the rDNA repeats.

(B) PFGE-Southern analyses of *SD5* using subtelomere DNA fragments covering all *SH* regions as probes. Genomic DNA was digested with NotI. Note that each *SH* region has a maximum size of 60 kb (Figure 1A). The remaining signals observed in *SD5* at the positions around the wells indicate the existence of *SH*-homologous regions at non-*SH* loci. Note that the sequence of the 17.5–19.5 kb region, *SH2R*, is 87% identical to that of chromosome 3 position 275342–277405, within the *mae2* gene locus in the *S. pombe* genome database (<http://www.pombase.org>).

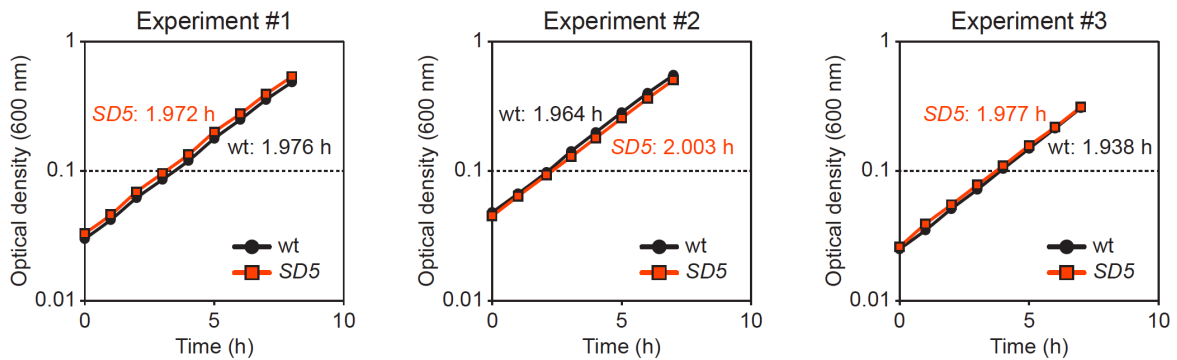
(C) Confirmation of complete isolation of the *SD5* mutant. The original *SD5* mutant and its three descendants after single colony isolation were analyzed for the 9-20 kb region of the subtelomere by PFGE-Southern. Genomic DNA was digested with NotI. The similar patterns of Southern signals were detected in all of the *SD5* strains, demonstrating that the *SD5* mutant used in this study is not contaminated with the wild-type strain.



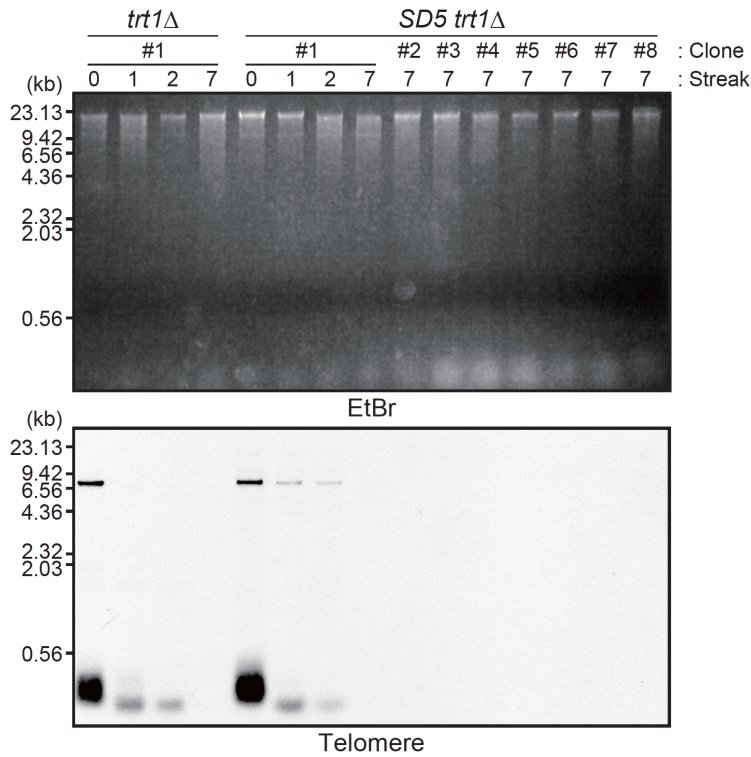
**Figure S2. Status of the marker genes in the *SD* mutants**

(A) The marker genes ( $ura4^+$  and  $his7^+$ ) used for the deletion of *SHs* are stably maintained. Genomic DNA of each strain was digested with MunI or PshB1 (the upper schematic diagram shows their target sites) and analyzed by Southern blots using *ura4*, *his7*, and telomere probes. The asterisk indicates the endogenous *his7* gene with a point mutation (*his7-366*).

(B) The marker genes used for the *SH* deletion are silenced. Cells were incubated on EMM+U+H (selective medium supplemented with uracil and histidine), EMM-U+H (lacking uracil), and EMM+U-H (lacking histidine) to examine auxotrophy for uracil and histidine. All of the media were supplemented with adenine, leucine, and lysine. In this assay, the endogenous *his7-366* gene was deleted because this mutant form tends to convert to the wild-type ( $his7^+$ ) form.

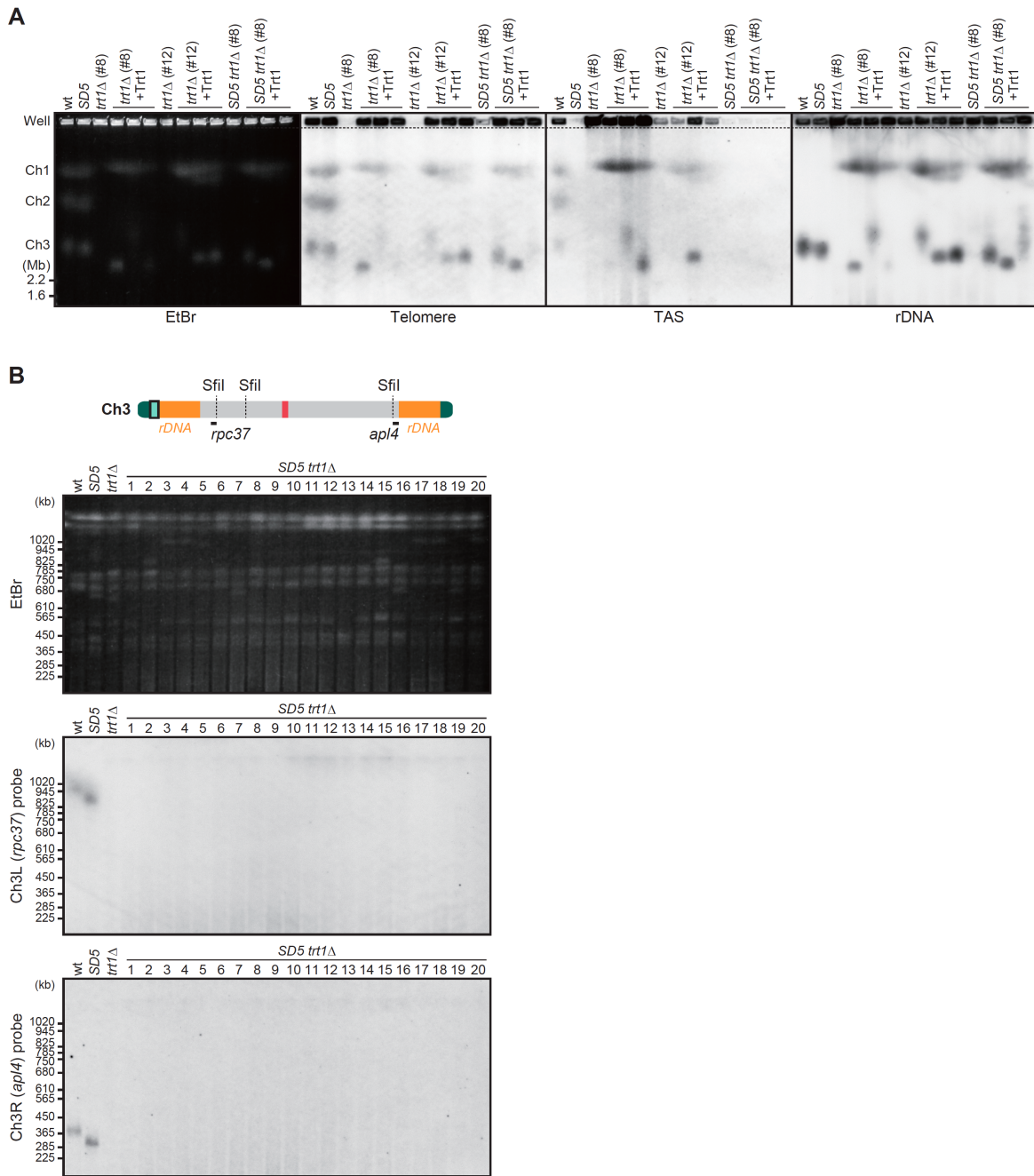


**Figure S3. *SD5* mutant cells proliferate at a rate similar to that of the wild-type strain**  
 Growth curves for wild-type (wt) and *SD5* strains. Cells were grown exponentially in YES liquid medium at 32°C, and the optical densities of cultures at 600 nm were measured every hour. Data from the three independent experiments are shown separately. Doubling times, estimated by the least-squares method, are indicated on each graph.



**Figure S4. Absence of telomere DNA in *SD5 trt1Δ* survivors**

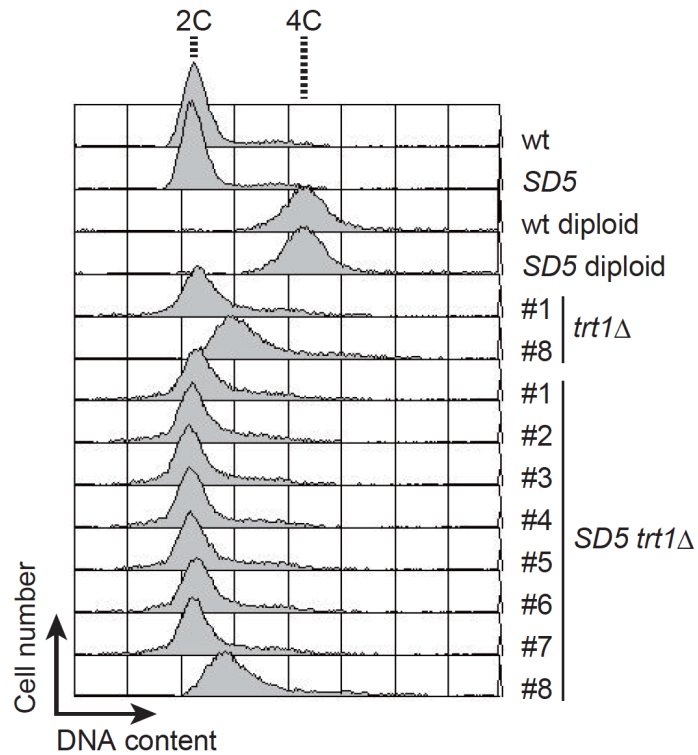
Southern blot analysis of *ApaI*-digested genomic DNA from wild-type and *SD5* strains after *Trt1* deletion. Genomic DNA was prepared from cells streaked 0, 1, 2, or 7 times on YES medium plates after deletion of *Trt1*. EtBr, ethidium bromide staining of the gel (upper). Telomere, Southern blot using telomere DNA as a probe (lower). Clone numbers correspond to those in Figures 3 and 4.



chromosomes to migrate into the gel, and amplified *SH* and/or rDNA were detected in the multiple chromosomes in three independent clones. TAS, a probe for TAS1-3 in *SH* (see Figure 1D).

(B) Ends of chromosome 3 of the survivors (types A, B, and C) exhibit a HAATI-type mode. PFGE-Southern analyses of *Sfi*I restriction fragments of genomic DNA *trt1*Δ (#1) and *SD5 trt1*Δ (#1-20). DNA sequences of the *rpc37* and *apl4* genes were used to detect rDNA-containing *Sfi*I fragments of *3L* and *3R*, respectively. The ends of chromosome 3 of these survivors did not migrate into the gel.





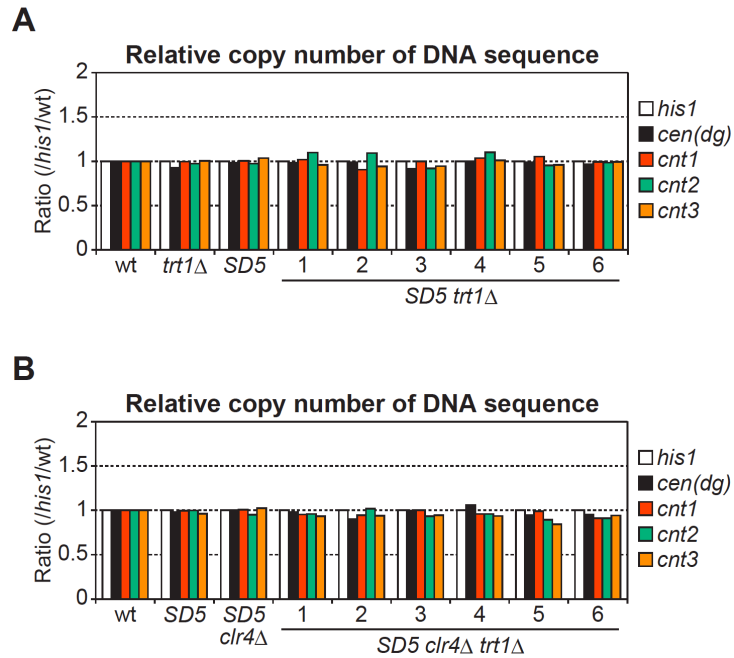
**Figure S6. *SD5 trt1Δ* survivors maintain a haploid state**

FACS (fluorescence activated cell sorting) analyses of exponentially growing cells of *trt1Δ* (#1 and 8) and *SD5 trt1Δ* (#1-8). Majority of *S. pombe* haploid cells are in G<sub>2</sub> phase, exhibiting a 2C DNA content, whereas wild-type diploid cells exhibit a 4C DNA content. Type C survivors (*trt1Δ*#8 and *SD5 trt1Δ*#8) showed a moderate increase of the DNA content most likely due to the amplification of *SH* and/or rDNA (see Figure S5A).

Clone #	Sequences at fusion sites	Genomic loci at fusion sites
<b>1</b>	1L-110.8 kb <span style="border: 1px solid black; padding: 0 2px;">TCAG</span> 1R-101.3 kb ACCTCGTTCC <span style="border: 1px solid black; padding: 0 2px;">TCAG</span> TTTAGTTATT	LTR
	2L-118.6 kb <span style="border: 1px solid black; padding: 0 2px;">GTTAT</span> ... (60 b) ...CTACT 2R-61.0 kb CCTCAGTTCA <span style="border: 1px solid black; padding: 0 2px;">GTTAT</span> ... (60 b) ...CTACTCATCAACCTG	LTR
<b>2</b>	1L-69.4 kb <span style="border: 1px solid black; padding: 0 2px;">CAAAGTAACTACG</span> 2L-84.1 kb ATGAACCGAG <span style="border: 1px solid black; padding: 0 2px;">CAAAGTAACTACG</span> TTTTTGCAAAA <span style="border: 1px solid black; padding: 0 2px;">AACTAAACAAG</span>	L-asparaginase (SPAC977.12, SPBPB8B6.05c)
	1R-147.5 kb <span style="border: 1px solid black; padding: 0 2px;">AAACATGG</span> 2R-72.2 kb CTTTATTACA <span style="border: 1px solid black; padding: 0 2px;">AAACATGG</span> GGTTCGAGTT	Phosphoprotein phosphatase (SPAC1039.02, SPBPB2B2.06c)
<b>3</b>	1L-110.5 kb <span style="border: 1px solid black; padding: 0 2px;">AGTATAGGTATTGTATTA</span> 2R-104.9 kb GTAGCAACTA <span style="border: 1px solid black; padding: 0 2px;">AGTATAGGTATTGTATTA</span> ACT <span style="border: 1px solid black; padding: 0 2px;">AGGTATAAAT</span>	LTR
	1R-61.4 kb <span style="border: 1px solid black; padding: 0 2px;">ACTTGTTAAATTA</span> 2L-107.2 kb ACGAAGTGG <span style="border: 1px solid black; padding: 0 2px;">ACTTGTTAAATTA</span> ACT <span style="border: 1px solid black; padding: 0 2px;">CTGGATAATG</span>	L-asparaginase (SPAC186.03, SPBPB21E7.09)
<b>4</b>	1L-110.5 kb <span style="border: 1px solid black; padding: 0 2px;">AGTATAGGTATTGTATTA</span> 2R-104.9 kb GTAGCAACTA <span style="border: 1px solid black; padding: 0 2px;">AGTATAGGTATTGTATTA</span> ACT <span style="border: 1px solid black; padding: 0 2px;">AGGTATAAAT</span>	LTR
	1R-62.2 kb <span style="border: 1px solid black; padding: 0 2px;">TTTTT</span> ... (557 b) ...TTCCT 2L-107.0 kb AACCGCGATG <span style="border: 1px solid black; padding: 0 2px;">TTTTT</span> ... (557 b) ...TTCCT <span style="border: 1px solid black; padding: 0 2px;">GCTTATTTC</span>	L-asparaginase (SPAC186.03, SPBPB21E7.09)
<b>5</b>	1L-110.5 kb <span style="border: 1px solid black; padding: 0 2px;">TAACTGAACTGAGGAACGAGGTT</span> 2R-105.0 kb ATATAGCTCA <span style="border: 1px solid black; padding: 0 2px;">TAACTGAACTGAGGAACGAGGTT</span> CAG <span style="border: 1px solid black; padding: 0 2px;">CAATAGCTCT</span>	LTR
	1R-61.6 kb <span style="border: 1px solid black; padding: 0 2px;">ACTGATATATTCAG</span> 2L-107.1 kb TCAAAACATT <span style="border: 1px solid black; padding: 0 2px;">ACTGATATATTCAG</span> TCTTGAGTAT	L-asparaginase (SPAC186.03, SPBPB21E7.09)
<b>6</b>	1L-110.5 kb <span style="border: 1px solid black; padding: 0 2px;">AGTTAT</span> 1R-101.4 kb TCCTCAGTTC <span style="border: 1px solid black; padding: 0 2px;">AGTTAT</span> TCACTATATC	LTR
	2L-118.6 kb <span style="border: 1px solid black; padding: 0 2px;">CTGAACCTCGTTCCTCAGTTC</span> 2R-61.0 kb AGAGCTACTG <span style="border: 1px solid black; padding: 0 2px;">CTGAACCTCGTTCCTCAGTTC</span> GGTTATGAGC	LTR
<b>7</b>	1L-110.8 kb <span style="border: 1px solid black; padding: 0 2px;">TCAGTTAT</span> 1L-110.6 kb 2L-118.5 kb GTTCCCTCAGC <span style="border: 1px solid black; padding: 0 2px;">TCAGTTAT</span> SAGCT... (105 b) ...CACCC <span style="border: 1px solid black; padding: 0 2px;">CAGTTCCTACG</span> <span style="border: 1px solid black; padding: 0 2px;">TATCCTTAAA</span>	LTR
	2L-106.4 kb <span style="border: 1px solid black; padding: 0 2px;">GTTTC</span> ... (258 b) ...AAAA 1R-62.5 kb AAATATCGCA <span style="border: 1px solid black; padding: 0 2px;">GTTTC</span> ... (258 b) ...AAAA <span style="border: 1px solid black; padding: 0 2px;">CGTAACATTG</span>	L-asparaginase (SPBPB21E7.09, SPAC186.03)
	2L-118.5 kb <span style="border: 1px solid black; padding: 0 2px;">AATCAGATACCAAACTGCGTAG</span> 2R-61.1 kb CGTATCCTTA <span style="border: 1px solid black; padding: 0 2px;">AATCAGATACCAAACTGCGTAG</span> CTTACAATAG	LTR

**Figure S7. DNA sequences of the intra- and inter-chromosomal end fusion sites in *SD5 trt1Δ* survivors (#1–7)**

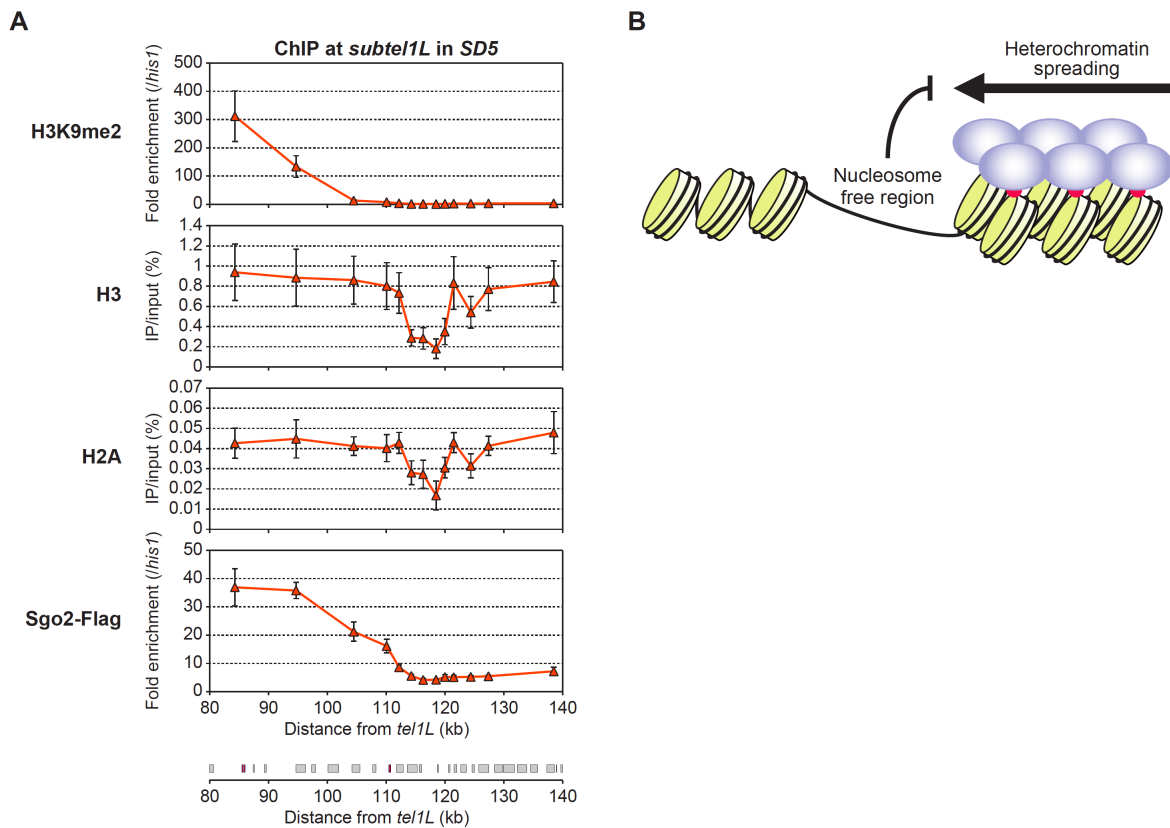
Letters in black and enclosed by a box indicate common sequences shared by two chromosome arms that were fused, and letters in blue, red, or orange indicate sequences specific to each arm. Distance from a fusion site to the nearest telomere is also indicated. The right column shows genomic loci at the fusion sites. LTR, long terminal repeat.



**Figure S8. Centromere DNA is normally present in *SD5 trt1*Δ and *SD5 trt1*Δ *clr4*Δ survivors**

(A) Relative copy numbers of genomic DNA sequences at each centromere region were analyzed by quantitative PCR. Values were normalized to that of the *his1* locus, and then compared with those of the wild-type strain. The strains presented in Figure 6A were analyzed.

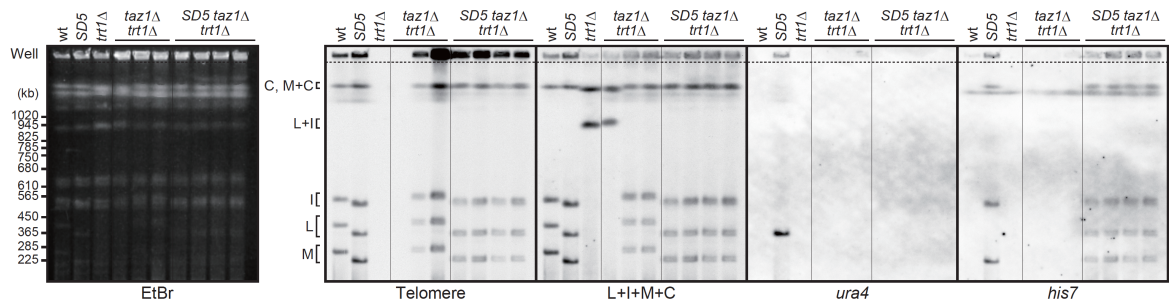
(B) The strains presented in Figure 6D were analyzed.



**Figure S9. Nucleosome-free configuration at the border of *subtel1L***

(A) ChIP analyses of the distributions of H3K9me2, total H3, total H2A, and Sgo2-Flag at 80–139 kb from *tel1L*. In the graphs of H3K9me2 and Sgo2-Flag, fold enrichment relative to the *his1* locus is shown. In the graphs for H3 and H2A, recovery of immunoprecipitated DNA relative to total input DNA is shown. Boxes below the graph indicate the ORFs of genes located in this region. *LTRs* are shown in magenta. Errors bars indicate the s. d. (n = 3).

(B) Model of subtelomeric chromatin boundary. Step-by-step heterochromatin spread may be blocked by the nucleosome-free configuration at the border of subtelomeres.



**Figure S10. *SH* is not required for cell survival by homologous recombination after the telomerase loss**

PFGE-Southern analyses of the Not1 fragments of chromosomes in survivors of *taz1Δ trt1Δ* or *SD5 taz1Δ trt1Δ*. The *trt1Δ* strain was used for a control of a survivor with circular chromosomes. Spores from diploid cells (*taz1*<sup>+/-</sup> *trt1*<sup>+/-</sup> with or without *SD5/SD5*) were dissected, and survivors after more than seven streaks were analyzed by PFGE-Southern using telomere, L+I+M+C (see Figure 1D), *ura4*, and *his7* as probes. Two of the three survivors of *taz1Δ trt1Δ* and all the four survivors of *SD5 taz1Δ trt1Δ* contained linear chromosomes 1 and 2, exhibiting L, I, M, and C fragments, whereas one *taz1Δ trt1Δ* survivor showed chromosome circularization, exhibiting L+I fragment. The asterisk indicates the endogenous *his7* gene with a point mutation (*his7-366*). Note that the *his7*<sup>+</sup> marker gene, which was used for the deletion of three *SH* regions (*SH1R*, *SH2L*, and *SH2R*), was detected with the *IL* fragment (L) as well as with the other fragments (I, M, and C), suggesting the possibility that the multiple *his7*<sup>+</sup> genes next to the telomeres were involved in the expansion of telomere DNA by homologous recombination. Interestingly, the *ura4*<sup>+</sup> marker gene used for the deletion of *SH1L* and *SH3L* was completely absent from the *SD5 taz1Δ trt1Δ* survivors, suggesting that dynamic rearrangements have occurred at the chromosome ends in these survivors.

## Supplementary Tables

**Table S1. Internal genes with significant changes of expression in *SD5***

Gene name	Systematic ID	Gene product	Ratio ( <i>SD5</i> /wt, log <sub>2</sub> )
<b>Chromosome 1</b>			
<i>pdc202</i>	SPAC13A11.06	pyruvate decarboxylase	-0.3
<i>urg2</i>	SPAC1002.17c	uracil phosphoribosyltransferase	-0.2
<i>urg1</i>	SPAC1002.19	GTP cyclohydrolase II	-0.3
	SPAC3H1.06c	transmembrane transporter	-0.3
	SPAC2E1P3.05c	fungal cellulose binding domain protein	-0.2
<i>fip1</i>	SPAC1F7.07c	iron permease	1
<i>fiol</i>	SPAC1F7.08	iron transport multicopper oxidase	0.8
	SPAC27D7.09c	But2 family protein	0.2
<b>Chromosome 2</b>			
<i>urh1</i>	SPBC1683.06c	uridine ribohydrolase	-0.3
	SPBC660.05	WW domain containing conserved fungal protein	-0.4
<i>fet4</i>	SPBP26C9.03c	iron/zinc ion transmembrane transporter	-0.2
	SPBC31A8.02		0.8
<i>isp4</i>	SPBC29B5.02c	OPT oligopeptide transmembrane transporter family	-0.2
	SPBC27.05		0.3
	SPBC1711.15c	<i>Schizosaccharomyces pombe</i> specific protein	0.3
	SPBC17G9.12c	hydrolase	0.5
<i>str1</i>	SPBC4F6.09	siderophore-iron transmembrane transporter	0.5
<i>spo6</i>	SPBC1778.04	Spo4-Spo6 kinase complex regulatory subunit	0.7
<i>htd2</i>	SPBC1105.15c	3-hydroxyacyl-ACP dehydratase	-0.4
<i>rpr2</i>	SPBC1105.16c	RNase P subunit	0.3
<i>met6</i>	SPBC56F2.11	homoserine O-acetyltransferase	-0.2
<b>Chromosome 3</b>			
<i>nic1</i>	SPCC1884.02	NiCoT heavy metal ion transmembrane transporter	-0.3
	SPCC18B5.02c		0.3
<i>ctr4</i>	SPCC1393.10	copper transporter complex subunit	-0.4
	SPCC338.18		0.4
<i>inv1</i>	SPCC191.11	external invertase, beta-fructofuranosidase	-0.4
<i>amt1</i>	SPCPB1C11.01	ammonium transmembrane transporter	-0.3

Genes indicated by blue shades are related to metal transport.

**Table S2. Fission yeast strains used in this study**

---

**Figure 1D**

JP1225	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366</i>
ST3579	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup></i>
ST3580	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1R::his7<sup>+</sup></i>
ST3582	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2L::his7<sup>+</sup></i>
ST3583	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2R::his7<sup>+</sup></i>
ST3593	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup></i>

**Figure 1F**

JP1225	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366</i>
ST3593	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup></i>
ST3585	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH3L::ura4<sup>+</sup></i>
ST3479	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup></i>

**Figures 2A and 2B**

JP1225	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366</i>
ST3479	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup></i>

**Figures 2C and 2D**

JP1224	<i>h<sup>+</sup> ade6-M210 leu1-32 ura4-D18 his7-366</i>
JP1225	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366</i>
ST3524	<i>h<sup>+</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup></i>
ST3479	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup></i>

**Figure 2E**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 JP3189 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 rad3::kan<sup>r</sup>*  
 ST2776 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 clr4::hyg<sup>r</sup>*  
 JP3204 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 taz1::hyg<sup>r</sup> rap1::ura4<sup>+</sup>*

**Figure 2F**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3579 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup>*  
 ST3580 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1R::his7<sup>+</sup>*  
 ST3582 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2L::his7<sup>+</sup>*  
 ST3583 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2R::his7<sup>+</sup>*  
 ST3585 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH3L::ura4<sup>+</sup>*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

**Figure 2G**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 YN2373 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 taz1::hyg<sup>r</sup>*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3482 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> taz1::hyg<sup>r</sup>*

**Figure 3**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 JP3689-3696 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1-8)*  
 JP3799-3810 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#9-20)*



**Figure 4**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1)*  
JP3697-3704 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-8)*  
JP3811-3822 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#9-20)*

**Figure 5B**

JP3697-3702 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-6)*

**Figures 5C and 5D**

JP3703 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#7)*

**Figures 6A and 6B**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1)*  
ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
JP3697-3702 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-6)*

**Figure 6C**

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
ST3499 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup>*  
JP3697-3699 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*

ST4381-4396      *SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-3)*  
*h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup> trt1::kan<sup>r</sup> (#1-16)*

**Figure 6D**

JP1225      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3499      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup>*  
 ST4381-4386      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup> trt1::kan<sup>r</sup> (#1-6)*

**Figure 7A**

JP1225      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3587      *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>*  
*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3589      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH2L::his7<sup>+</sup>*  
*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3590      *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3591      *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST3593      *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>*

**Figures 7B and 7C**

JP1225      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479      *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

**Figure 8B**

ST3591 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST4213 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> subtel2R(52.3-74.8kb)::nat<sup>r</sup>*

**Figure 8C**

ST3591 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST4213 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> subtel2R(52.3-74.8kb)::nat<sup>r</sup>*

ST4839 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH3L::ura4<sup>+</sup> sgo2-3flag-kan<sup>r</sup>*

ST4841 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> sgo2-3flag-kan<sup>r</sup>*

ST4843 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> subtel2R(52.3-74.8kb)::nat<sup>r</sup>  
sgo2-3flag-kan<sup>r</sup>*

**Figures 9A and 9B**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

**Figures 10A and 10B**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST2776 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 clr4::hyg<sup>r</sup>*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST3499 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup>*

**Figure 10C**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST2776 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 clr4::hyg<sup>r</sup>*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

ST3499 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup>*

ST3183 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 nhe1::kan<sup>r</sup>*

JP3560 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 spc1::kan<sup>r</sup>*

**Table 1**

ST4658,4659 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18  
his7-366/his7-366 SH1L::ura4<sup>+</sup>/SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>/SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup>/SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>/SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>/SH3L::ura4<sup>+</sup>  
trt1<sup>+</sup>/trt1::kan<sup>r</sup> rad51<sup>+</sup>/rad51::hyg<sup>r</sup> (#1,2)*

ST4661,4662 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18  
his7-366/his7-366 SH1L::ura4<sup>+</sup>/SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>/SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup>/SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>/SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>/SH3L::ura4<sup>+</sup>  
trt1<sup>+</sup>/trt1::kan<sup>r</sup> rad52<sup>+</sup>/rad52::hyg<sup>r</sup> (#1,2)*

**Figure S1A**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST3654 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 rpc37-kan<sup>r</sup>-NotI-rpl2402 (#1)*

ST3655 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 rpc37-kan<sup>r</sup>-NotI-rpl2402 (#2)*

ST3656 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 rpc37-kan<sup>r</sup>-NotI-rpl2402 (#3)*

ST3657 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 ppk38-kan<sup>r</sup>-NotI-mug170 (#1)*

ST3658 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 ppk38- kan<sup>r</sup>-NotI-mug170 (#2)*

ST3659 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 ppk38- kan<sup>r</sup>-NotI-mug170 (#3)*

### Figures S1B and S1C

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

### Figure S2A

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST3579 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup>*

ST3580 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1R::his7<sup>+</sup>*

ST3582 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2L::his7<sup>+</sup>*

ST3583 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH2R::his7<sup>+</sup>*

ST3593 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>*

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*

ST3593 *h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>*

ST3585 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH3L::ura4<sup>+</sup>*

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

### Figure S2B

ST5171 *h<sup>+</sup> ade6-M210 leu1-32 ura4-D18 his7::BSD*

ST5173 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7::BSD*

ST5175 *h<sup>+</sup> ade6-M210 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> his7::BSD*

ST5177 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> his7::BSD*

JK107 *h<sup>-</sup>*

**Figure S3**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

**Figure S4**

JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1)*  
 JP3697-3704 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
 SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-8)*

**Figure S5A**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
 SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 JP3696 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#8)*  
 ST5125-5127 *h<sup>-</sup> ade6-M216 ura4-D18 his7-366 trt1::kan<sup>r</sup> leu1::trt1<sup>+</sup>-hyg<sup>r</sup> (#8-1, 2, 3)*  
 JP3802 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#12)*  
 ST5128-5130 *h<sup>-</sup> ade6-M216 ura4-D18 his7-366 trt1::kan<sup>r</sup> leu1::trt1<sup>+</sup>-hyg<sup>r</sup> (#12-1, 2, 3)*  
 JP3704 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
 SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#8)*  
 ST5131-5133 *h<sup>-</sup> ade6-M216 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>  
 SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> leu1::trt1<sup>+</sup>-hyg<sup>r</sup> (#8-1, 2, 3)*

**Figure S5B**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
 SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1)*  
 JP3697-3704 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
 SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup> (#1-8)*  
 JP3811-3822 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>*

*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup>* (#9-20)

**Figure S6**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
ST3526 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18  
his7-366/his7-366*  
ST3527 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18  
his7-366/his7<sup>+</sup> SH1L::ura4<sup>+</sup>/SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>/SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup>/SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>/SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>/SH3L::ura4<sup>+</sup>*  
JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup>* (#1)  
JP3696 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup>* (#8)  
JP3697-3704 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup>* (#1-8)

**Figure S7**

JP3697-3703 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup>* (#1-7)

**Figure S8A**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup>* (#1)  
ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
JP3697-3702 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>  
SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> trt1::kan<sup>r</sup>* (#1-6)

**Figure S8B**

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*

ST3499 *SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
*h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup>*  
 ST4381-4386 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> clr4::hyg<sup>r</sup> trt1::kan<sup>r</sup> (#1-6)*

#### Figure S9A

ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 ST4841 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> sgo2-3flag-kan<sup>r</sup>*

#### Figure S10

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*  
 ST3479 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>*  
*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*  
 JP3689 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 trt1::kan<sup>r</sup> (#1)*  
 ST5180-5182 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366 taz1::hyg<sup>r</sup> trt1::kan<sup>r</sup> (#2-4)*  
 ST5183-5186 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>*  
*SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup> taz1::hyg<sup>r</sup> trt1::kan<sup>r</sup> (#1-4)*  
 ST5134 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18*  
*his7-366/his7-366 taz1<sup>+</sup>/taz1::hyg<sup>r</sup> trt1<sup>+</sup>/trt1::kan<sup>r</sup>*  
 ST5138 *h<sup>+</sup>/h<sup>-</sup> ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18*  
*his7-366/his7<sup>+</sup> SH1L::ura4<sup>+</sup>/SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup>/SH1R::his7<sup>+</sup>*  
*SH2L::his7<sup>+</sup>/SH2L::his7<sup>+</sup> SH2R::his7<sup>+</sup>/SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>/SH3L::ura4<sup>+</sup>*  
*taz1<sup>+</sup>/taz1::hyg<sup>r</sup> trt1<sup>+</sup>/trt1::kan<sup>r</sup>*

#### Table S1

JP1225 *h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 his7-366*



ST3479

*h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 SH1L::ura4<sup>+</sup> SH1R::his7<sup>+</sup> SH2L::his7<sup>+</sup>  
SH2R::his7<sup>+</sup> SH3L::ura4<sup>+</sup>*

**Table S3. Primers used for strain construction**

---

***rad3* deletion (PCR-based)**

cs125 5'-AGCTGGGTCACCTCGATCTTTC-3'  
cs126 5'-GGGGATCCGTCGACCTGCAGCGTACGAAAGTCCACGCTGTAAAGAGAG-3'  
cs127 5'-GTTTAAACGAGCTCGAATTCATCGATTGTTGAATCTTTCCAGAACC-3'  
cs128 5'-AGCCCAACCAATGTACATTTTC-3'

***clr4* deletion (PCR-based)**

jk1467 5'-AGTAATATCCTAACATATGACATATAG-3'  
jk1468 5'-GGGGATCCGTCGACCTGCAGCGTACGACGCAAACTAATAACCTCTTGTGTTG-3'  
jk1469 5'-GTTTAAACGAGCTCGAATTCATCGATCCACTCCTGTTACCTAATATAGC-3'  
jk1470 5'-CTTCACATGTGCGCAGCAGATG-3'

***taz1* deletion (PCR-based)**

jk969 5'-TTGTTTCAGTTTGACGTTCTTGTG-3'  
jk970 5'-GGGGATCCGTCGACCTGCAGCGTACGATCTGTACTTTGCACGCTTATCATC-3'  
jk967 5'-GTTTAAACGAGCTCGAATTCATCGATGTCTCCGGATAGAGTTTGTTCATG-3'  
jk968 5'-TTGTGCTTCACTCATTACGATTCC-3'

***rap1* deletion (PCR-based)**

if1 5'-TTCATAACAATGATGAATTCTGAA-3'  
if2 5'-TTAATTAACCCGGGGATCCGAGTTCATACTTTTATTCTTTTTTGC-3'  
jk1209 5'-GTTTAAACGAGCTCGAATTCATCGATCCGAGCATCTCTGCCCAAATTC-3'  
jk1210 5'-ATGAAGAATCTGGAGTTGACGTAG-3'

***nhe1* deletion (PCR-based)**

st55 5'-TATTGAGATAAAAATGCCTTAATCCAATCAG-3'  
st56 5'-GGGGATCCGTCGACCTGCAGCGTACGAAGTAATTAGGCAATATCCACAAGAG-3'  
st57 5'-GTTTAAACGAGCTCGAATTCATCGATTAGGAAAGCTCTTTAATGTCAATTC-3'  
st58 5'-TCCGTTTTCTTAGTTTTCTTACATAAAAATAG-3'

***spc1* deletion (PCR-based)**

jk1623 5'-GTTGATCTAATAAAGCTGGCTTAAC-3'  
jk1624 5'-GGGGATCCGTCGACCTGCAGCGTACGATTTATTCAAACCTGGTTACAAAAAGGAC-3'

jk1625 5'-GTTTAAACGAGCTCGAATTCATCGATACAAGTTGTAGATAAAGCCTTAAAAG-3'

jk1626 5'-TTATAAAACCCGTAACCTACATTAACG-3'

***trt1* deletion (PCR-based)**

jk1631 5'-GGTATCATCCAACGTTTGCCTTG-3'

jk1632 5'-GGGGATCCGTCGACCTGCAGCGTACGAGCAAGTTACAAGTAACCATTAAGCA-3'

jk1633 5'-GTTTAAACGAGCTCGAATTCATCGATGTCATTTTCAATTTATTATATACATCC-3'

jk1634 5'-GTTTCTTTTCGTCTCAGTAGCAAG-3'

***rad51* deletion (PCR-based)**

jk1318 5'-GAATAAGACGACTACACGCTATGG-3'

jk1319 5'-GGGGATCCGTCGACCTGCAGCGTACGATAACTTGTTAAGCACGAAATTATCAC-3'

jk1320 5'-GTTTAAACGAGCTCGAATTCATCGATTTGCAAATTTCTATGGGATGAGTTTG-3'

jk1321 5'-CAAGAATTTAGCATTTTGCCTGCG-3'

***rad52* deletion (PCR-based)**

st381 5'-CATTAGAAGGTTCTGAAGAAGTATGG-3'

st382 5'-GGGGATCCGTCGACCTGCAGCGTACGACCCTTCTAGCTTATATGAAGCAGT-3'

st383 5'-GTTTAAACGAGCTCGAATTCATCGATTAAGTAATGAGGCAAAATGTGA-3'

st384 5'-AAACGACTTCCGCGATAAAG-3'

**NotI insertion in Ch3L (PCR-based)**

st179 5'-CAATTTGATGGTCCGGTCTAATTC-3'

st180 5'-GGGGATCCGTCGACCTGCAGCGTACGACATCTGTAAGTAAATACGACTTACTCTTG-3'

st181 5'-GTTTAAACGAGCTCGAATTCATCGATGCGGCCCTAACATGATGACTGAAAATATTGACG-3'

st182 5'-GTTTGTCATGGACTGTCCTTTAC-3'

**NotI insertion in Ch3R (PCR-based)**

st183 5'-CCTGTAAACTTAAAATCTGTGGAAAAC-3'

st184 5'-GGGGATCCGTCGACCTGCAGCGTACGAAAACGGTAACTTAGTTGGTAAAAAAAC-3'

st185 5'-GTTTAAACGAGCTCGAATTCATCGATGCGGCCGCTGTTTTGGAATGTTTACCATCGAG-3'

st186 5'-CTTTATCCAAACACCGTTTCC-3'

***subtel2R(52.3–74.8kb)* deletion (PCR-based)**

km33 5'-GACGACGGACTTTGTGAAGGA-3'

st73 5'-GGGGATCCGTCGACCTGCAGCGTACGAGATTAACGAAATAGATTAGAATTGC-3'

st22 5'-GTTTAAACGAGCTCGAATTCATCGATTCCCGCTGATAAACATCGATTCAC-3'  
jk700 5'-TCCGCAAATTTGTTAGCCAT-3'

**Table S4. Primer sets used for quantitative PCR**

---

***his1***

jk1335 5'-CGAAGACGTGCTTCAGCGA-3'

jk1336 5'-TGTCCACCTCGGAATCACTG-3'

***ade6***

st15 5'-GAAAGATGCTGCCGTCATTTTAG-3'

st16 5'-GCTGCGGTACGAGCATAAGTAAC-3'

***cen(dg)***

st17 5'-AATTGTGGTGGTGTGGTAATAC-3'

st18 5'-GGGTTCATCGTTTCCATTCAG-3'

***cnt1***

st334 5'-CCTTCTGGAGTTCTTTGGACCTACT-3'

st335 5'-CAAACGGAGGACGATGAACTAAT-3'

***cnt2***

st323 5'-ACTAATAACGGAATAGATCAAACAAGCTC-3'

st324 5'-TATTTAACCAGCAAATTCATAGATTTGAC-3'

***cnt3***

st325 5'-AGCATTCGATAATTTGAGCTTTAATG-3'

st326 5'-AAAATGTGAATGTAAGGCCGTTCT-3'

***tlh***

jk457 5'-CACTCATTTCTTTGCTGATCACTTACT-3'

jk458 5'-TCGATAGAGATACGTTTCATCTCATAGTGT-3'

***rDNA***

TB19 5'-GGGAACCAGGACTTTTACCTTGA-3'

TB20 5'-AACTTGCCTGCTTTGAACACTCTA-3'

***subtel-2.8 kb***

jk614 5'-GTCTCGTTGCTCGCTTCACA-3'

jk615 5'-GGAGGATGGGAAATTTGAGGAT-3'

***subtel-9.0 kb***

jk620 5'-TTCTTAATCATTATCAAGTATTCATTGCAA-3'  
 jk621 5'-ACAGTAAACTATGATCGCTTTTGAAGAC-3'  
**subtel-14.7 kb**  
 jk1607 5'-GCTTGGTCAACATCATCTTGTGCG-3'  
 jk1608 5'-GGGAGCAGCAAGACAAAGGTG-3'  
**subtel-20.0 kb**  
 jk455 5'-AACGAGTTGTGCAATGTTAGTAAGGT-3'  
 jk456 5'-GACCGCTACGCAACCATAAAG-3'  
**subtel-27.2 kb**  
 jk554 5'-GCTGCATGTTTATATCCTTGGCT-3'  
 jk555 5'-GACCCCAATGGCCGAGAT-3'  
**subtel-30.0 kb**  
 jk388 5'-GGCACAATTTTCATTTTCGTTTAGTTTAC-3'  
 jk389 5'-TGTTACTCATACTTGAAATACAATTTGAATG-3'  
**subtel-33.7 kb**  
 jk686 5'-GCATTCCAGCAGAACCAGGT-3'  
 jk687 5'-GCAAACACCCACCCTGAAGTA-3'  
**subtel-37.9 kb**  
 jk690 5'-TGAAACGGGTTCTTACTGCGT-3'  
 jk691 5'-GCTCCATCCATTGTCATTGGT-3'  
**subtel-41.6 kb**  
 jk692 5'-CGCGCACTTTTTCGGACATA-3'  
 jk693 5'-TGCAAGTCGCCGAAACTACC-3'  
**subtel-45.7 kb**  
 jk696 5'-TTGAATCCCTCATCCAAAGGA-3'  
 jk697 5'-TTGGTGTGTCAGCCCATTGAACT-3'  
**subtelLL-65.0 kb (nheI)**  
 yn114 5'-TCCGGACATTAAGACATGGAAA-3'  
 yn115 5'-CAAACCCCTATTGGTCCGAAA-3'  
**subtelLL-74.8 kb**

yn116 5'-TCCCTCCAATGTCTTTCAGCAT-3'  
 yn117 5'-GGCCCCTACGAACCGTAATT-3'  
**subtelLL-84.3 kb**  
 yn118 5'-GCTGATGTTGCACCAGTTCAA-3'  
 yn119 5'-GAAACAACCTATTCAGCCGATGCT-3'  
**subtelLL-94.7 kb**  
 yn120 5'-TTTATCTCAATGTCCGGATGGTT-3'  
 yn121 5'-CCAAGATGCCGCTAATGGAA-3'  
**subtelLL-104.5 kb**  
 yn122 5'-CGATTTTCTCAGCGGGAAC-3'  
 yn123 5'-TGGCGTCTTTGCTTCCAAA-3'  
**subtelLL-110.1 kb**  
 st221 5'-ACGGCAGAGGTATACAAATAATCATAAG-3'  
 st222 5'-CCCGAACGTATATCATCGCAAT-3'  
**subtelLL-112.2 kb**  
 st429 5'-AACATTCGTTTCTTCATCCACAAC-3'  
 st430 5'-TCTAATCCACGAACTCCATCACA-3'  
**subtelLL-114.3 kb**  
 st94 5'-TGGCAAGAAGCTCCAAGAATTC-3'  
 st95 5'-AGCGTTTCCATCCCTGGTTA-3'  
**subtelLL-116.3 kb**  
 st431 5'-TCATTATCTTTGGTGTGTGCAAGA-3'  
 st432 5'-CTCGGAGCTCATCTGTAAAGCTT-3'  
**subtelLL-118.5 kb**  
 st433 5'-AATGGCTGATTGGCTACTGATTAAT-3'  
 st434 5'-CCCGTCTTCAAAGGAATCAATT-3'  
**subtelLL-120.0 kb**  
 st457 5'-CATAGCATTGCACAATCTCTCGAT-3'  
 st458 5'-TCACAAGAAGGCCTATTCAATCC-3'  
**subtelLL-121.5 kb**

st447 5'-AAATGGTGTTAAGCGGTTATTCG-3'

st448 5'-GATGGAATACCCCATGTGGAAA-3'

**subtelLL-124.4 kb**

st96 5'-AACAATGGGACACTTCGCAATTAAC-3'

st97 5'-GGGAATCTCGATTTGACATAATGA-3'

**subtelLL-127.4 kb**

st449 5'-TTCTGTAGCGATGGTTCCCAT-3'

st450 5'-CTCCACGCACGAATGCTCT-3'

**subtelLL-138.5 kb**

st455 5'-GGCCTCCCCAAAGGATTG-3'

st456 5'-ATTGCTTCGTCAGAAATCCCA-3'

**subtelLR-40.7 kb**

jk714 5'-AATTAATTCGGTGCATCGTGC-3'

jk715 5'-GATGCAACGTTTCATTTGCGT-3'

**subtelLR-50.5 kb**

jk724 5'-TTCCGCTACAACCCGTTTAAA-3'

jk725 5'-AAAGCGATAGCATAAGTGTATTCGAA-3'

**subtelLR-60.6 kb**

jk734 5'-TCGTTTTGGTGCCGCTAAAT-3'

jk735 5'-AATAATGGTCACTAGATGTGCGGTT-3'

**subtelLR-72.5 kb**

jk746 5'-AAACAAAGCAGATTCCCACAGAA-3'

jk747 5'-TCAAACCAAGTTTGCTCACCC-3'

**subtelLR-82.3 kb**

jk752 5'-GTTTATGGGAACAGCTGATGCAT-3'

jk753 5'-AAGGCATTTTTGCATATTTGTGC-3'

**subtelLR-92.4 kb**

jk756 5'-TTTTACCACAGTACTTGATCACGAATTT-3'

jk757 5'-TTATGCAAGAGTTCTGTGATTTGAAA-3'

**subtel2L-63.1 kb**



yn124 5'-CACCCCAAGTAACCAAATATGGA-3'  
 yn125 5'-CGACTGACAACAAAAGCGATTTT-3'  
**subtel2L-72.5 kb**  
 yn126 5'-TGAAC TTTCCGCTCGTGAAA-3'  
 yn127 5'-TTCTTGGCTGAATGAGAAAGTTGT-3'  
**subtel2L-83.1 kb**  
 yn128 5'-ACTGGCTTGAGTAGAAGGTGCTTT-3'  
 yn129 5'-CCACACTCGGTTGTAAGATCATCA-3'  
**subtel2L-92.5 kb**  
 yn130 5'-ATCTACCTATGGATTCGGTTTAGCA-3'  
 yn131 5'-CTACTTGACGACGACGAATCTGA-3'  
**subtel2L-102.8 kb**  
 yn132 5'-TGCAATCAAAGGACCGAATAAAA-3'  
 yn133 5'-TGCAAAAAATGCTGGCGTTA-3'  
**subtel2L-109.8 kb**  
 st177 5'-AAAAAGGGATATTTGTTTCAGCAGAAC-3'  
 st178 5'-ATGAAAATTCTGACGAAGAAGATGAG -3'  
**subtel2R-51.8 kb**  
 jk700 5'-TCCGCAAATTTTGTAGCCAT-3'  
 jk701 5'-AGCTTAATTCGTGATGCAAGTTTTTA-3'  
**subtel2R-61.7 kb**  
 km20 5'-CACTTACGCCATAGTGGATTGC-3'  
 km21 5'-CGAAAGACTGACTAAGCGAATCG-3'  
**subtel2R-71.6 kb**  
 km28 5'-CCCGACTCCCTCAACATGAA-3'  
 km29 5'-CAAAGCACAAGGGTTGACCA-3'  
**subtel2R-79.8 kb**  
 km36 5'-CGGTTACTAGTGCCACTGCATT-3'  
 km37 5'-CCATTTAAGTAGTTGCGGCGA-3'  
**subtel2R-89.8 kb**

st110 5'-TGCGGACATCATAACATGCTAAA-3'  
 st111 5'-TTCAATATACACCGAGGTCCGAAT-3'  
**subtel2R-92.8 kb**  
 st80 5'-CGTATGAAGTGCTACGATGAAACA-3'  
 st81 5'-GCATGGTTTTGGGATGATTTAGA-3'  
**subtel2R-93.8 kb**  
 st84 5'-CCGTTTCGTGTGAATGGTACCT-3'  
 st85 5'-CACAGCACTAGCATTGCAAAGAG-3'  
**subtel2R-94.9 kb**  
 st86 5'-TCAAAGACATGGTTCTCGTAATCC-3'  
 st87 5'-GCGATTCCAACAGCATCGA-3'  
**subtel2R-95.9 kb**  
 st82 5'-CATATCGAAGGTCAGCGTCTAAAA-3'  
 st83 5'-TCCCCAGACCAAGATATAATTGTTTAA-3'  
**subtel2R-98.9 kb**  
 km38 5'-TTCAGGATTAAGGTAACGCGGT-3'  
 km39 5'-TTGCAGTTGTCCGCTAGTGC-3'  
**subtel2R-100.3 kb**  
 st459 5'-GCGTGTGAATTCGGCAATATAC-3'  
 st460 5'-CGTGATCAGTCAAGGGCAGTT-3'  
**subtel2R-101.6 kb**  
 st461 5'-GCTAAATGAGCTCAGGATTGAAGAA-3'  
 st462 5'-CTTTTATCACCCCTACTAATTGCATTAT-3'  
**subtel2R-103.5 kb**  
 st104 5'-TTTATGCATCCAACCGTTTCGA-3'  
 st105 5'-TTTCAATTGTCAGTTTGCCTATATATGG-3'  
**subtel2R-107.1 kb**  
 st213 5'-GCGACAAATGTGAATCAGTGTCTAT-3'  
 st214 5'-GCTTTACTCCTTTCAGGCGTATG-3'  
**subtel2R-110.8 kb**

km40            5'-CCTATGCCTACGCATTCAGCTC-3'  
 km41            5'-CGATCGTCCACTACTTCACGTTT-3'  
***subtel2R-122.8 kb***  
 st106           5'-AATTTTACCGGCTTCGCATCT-3'  
 st107           5'-TGCGTTTTCTCCGTGAATGA-3'  
***subtel2R-135.1 kb***  
 st215           5'- GCAGCTCTATTTGTCTCATAACGTTTAA-3'  
 st216           5'- CAGAACTTTGATTGCGGTAATTGA-3'  
***subtel2R-149.9 kb***  
 st112           5'-TCTACTCTGCTATCCACTAATTCCGTAT-3'  
 st113           5'-TTTCAATCAAAACATCGCATCTTAC-3'  
***subtel3L-15.9 kb***  
 st153           5'-CCAGTCAGCCATCGCTTATCA-3'  
 st154           5'-TTTCGTATGGCTGGTCTATTTAC-3'  
***subtel3L-16.8 kb***  
 st155           5'-CGTTGATTCTCATCGAAATACGTT-3'  
 st156           5'-GGTTGTTTTGGCATGTCCTATCT-3'