Supplementary Methods and Figure Legends

Supplementary methods

Strains and media

Strains used for this study are listed in Table S1. Gene deletions and tag insertions were created by the one-step gene insertion method [1]. The kanMX6 cassette was, as the need arose, switched to hygMX6 (HphMX6) [2, 3], his3+ (his3+ MX6) or leu1+ (kanMX6::leu1+) [4]. For replacements with ura4, fragments were amplified from pKS-ura4 [5]. Multiply mutated genotypes were derived by mating as appropriate. All media were as described [6]. Vegetatively growing cells (32°C) were plated on malt extract media at 30°C to induce meiosis. Live analysis was carried out approximately 7 hours after meiotic induction.

The ‘synthetic telomere insert’ was created by tandemly ligating annealed 49bp oligonucleotides (GGGTTACGGGTTACGAGGGTTACGAGGGTTACAGGGGTAC A and its complement) and cloning a gel-purified ligation product (of ~500 bp) upstream of the ScLEU2 gene in plasmid pIRT2 to create the TELO plasmid [7]; the synthetic telomere insert along with ScLEU2 was subcloned into the ura4 sequence in plasmid pBlueScript to create the template for amplification of the synthetic telomere for genomic integration. To create the strain harboring the internal telomere, this cassette was integrated into the genomic ura4 locus on chromosome III by transformation and selection for 5-Fluoroorotic Acid (5-FOA) resistant clones.

<table>
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<tr>
<th>Name</th>
<th>JCF Nº</th>
<th>Mating type</th>
<th>Genotype</th>
<th>Figure</th>
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<td>wt</td>
<td>5417</td>
<td>h&lt;sup&gt;90&lt;/sup&gt;</td>
<td>leu1-32 ura4-D18 his3-D1 lys1: Pnmt1: GFP-atb2 hht1-mRFP: kanMX6:: leu1+ sid4- GFP: kanMX6</td>
<td>1A, 2D S3</td>
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<td>‘linear’</td>
<td>7158</td>
<td>h&lt;sup&gt;90&lt;/sup&gt;</td>
<td>ade6-M210 leu1-32 lys1: Pnmt1: GFP-atb2 hht1-mRFP: kanMX6:: leu1+ sid4-GFP: kanMX6 bqt1:: hygMX6</td>
<td>1B</td>
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<td>Circular Type B</td>
<td>6914</td>
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<td>ade6-M210 leu1-32 his3-D1 taz1-YFP: kanMX6 hht1-CFP: hygMX6 sid4-mCherry: natMX6 trt1::his3+ atb2: Pnmt1: mRFP-atb2-LEU2 ade6-M210 leu1-32 his3-D1 taz1-YFP: kanMX6 hht1-CFP: hygMX6 sid4-mCherry: natMX6 trt1::his3+</td>
<td>1C-E, 2D, S5A</td>
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<td>ade6-M210 leu1-32 his3-D1 tat1-YFP: kanMX6 hht1-CFP: hygMX6 sid4-mCherry: natMX6 trt1::his3+</td>
<td>S1B, S3</td>
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<td>2B-D S3</td>
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<td>4A-D, S1D, S2B, S3</td>
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<td>3A-C</td>
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<td>2D, S4B</td>
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**Live analysis**

Cells were adhered to 35mm glass culture dishes (MatTek) precoated with 0.2 mg/ml soybean lectin (Sigma) and immersed in EMM-N with required supplements (plus 15 mM thiamine for strains harbouring *Pnmt1-GFP-atb2* or *Pnmt1-mRFP-atb2* constructs). Time-lapse live cell imaging was carried out in a temperature controlled Environmental Chamber set at 28°C with a DeltaVision Spectris (Applied Precision) comprising an Olympus IX70 wide-field inverted epifluorescence microscope, an Olympus UPlanSapo 100x NA 1.4 oil immersion objective, and a Photometrics CCD CoolSnap HQ camera. Images were analysed using SoftWoRx (Applied Precision). Images were acquired at 0.35µm step size over 26 focal planes; this was repeated every 10 min for 7 hr. wt, bqt1Δ, and *trt1Δrec12Δ* movies were captured with 0.2
second exposure per plane for the GFP channel and 0.06 sec/plane for the RFP channel, while trt1\(\Delta\) (type A or B), trt1\(\Delta\) with internal telomere, and trt1\(\Delta\) with the telomeric plasmid were captured with 0.3 sec/plane for the RFP and YFP channels and 0.2 sec/plane for the CFP channel. Images were deconvolved and combined to form a 2D image using the Maximum Intensity projection setting for esthetic purposes and Sum Intensity projection setting for quantitation purposes.
**Supplementary Figure Legends**

**Figure S1.** Taz1 localizes to wt telomeres as well as inserted chromosomal or plasmid-borne telomere stretches. The top of each vertical row depicts the sites of telomeres in the strains imaged in the panels below (‘chromatin’ is viewed via Hht1-CFP; Taz1 is endogenously tagged with YFP and indicated as ‘telomere’). (A) In wt mitotically growing cells, Taz1 localize to 2 to 4 foci. (B) In circular \textit{trt1}Δ strains, Taz1 shows diffuse nuclear localization during mitotic interphase. A single Taz1 focus is seen at the SPB in meiotic circular strains, as expected based on the ability of Taz1-A606V, which lacks DNA binding ability, to associate with the meiotic SPB; ref. (C and D) Discrete foci are observed in strains harbouring telomere repeat stretches on Chr III or a plasmid (C and D, respectively). Scale bars (to the left of each merged image) represent 5 µm.

**Figure S2.** (A–C) Internally placed telomere stretches successfully associate with the SPB during meiotic prophase, but fail to do so in a \textit{bqt1}Δ background (D). Color designations are as described in Figure S1. Scale bars (to the left of each bottom row) represent 5 µm.

**Figure S3.** Quantitation of meiotic SPB division and separation defects in the strain backgrounds indicated.

**Figure S4.** Circular strains with an internal telomere stretch do not rescue meiotic SPB and spindle defects when bouquet formation is disrupted. Color designations are as described for Figure 1. Scale bars (to the left of each row) represent 5 µm. (A) Strains lacking Bqt1 fail to form contacts between the SPB and the internal telomere stretch during meiotic prophase and fail in bipolar spindle formation at both MI and MII. (B) The internal telomere stretch also fails to rescue SPB division and spindle formation if the gene encoding Rap1 is deleted. Quantitation is shown in Figure 2D.

**Figure S5.** Severely entangled circular meiotic chromosomes and segregation defects are partially resolved by \textit{rec12}+ deletion. (A) Even in those circular \textit{trt1}Δ strains that form good spindles, chromosomes appear entangled and ‘sticky’ (see text). (B–D) Deletion of \textit{rec12}+ confers substantial rescue of the ‘stickiness’ of meiotic circular chromosomes. Asterisks indicate lagging chromosomes. Hence, recombination is largely responsible for the chromosome entanglement observed in meiotic circular strains. Scale bars (to the left of each row) represent 5 µm.
Supplementary References


Figure S1

A. Diagram showing chromosomes Chr I, Chr II, and Chr III with a representation of cen and telo regions.

B. Chromatin staining in WT, Otrt1Δ, and Otrt1Δ + Internal TELO conditions.

C. Taz1 staining in WT, Otrt1Δ, and Otrt1Δ + Internal TELO conditions.

D. Merge of Chromatin and Taz1 staining in WT, Otrt1Δ, and Otrt1Δ + TELO plasmid conditions.

Legend:
- WT: Wild Type
- Otrt1Δ: Deletion of Otrt1
- TELO: Telomere repeats
- Internal TELO: Internal region of TELO
- Telo repeats: Telomere repeats

Note: Images show fluorescence microscopy results.
FIGURE S2

A. Otrt1Δ + Internal TELO

B. Otrt1Δ + TELO Plasmid

C. Otrt1Δ pot1Δ + Internal TELO

D. Otrt1Δ bqt1Δ + Internal TELO

Merge

Chromatin

SPB & Tubulin

Taz1

-40 -20 -10

-100 -40 -20

-70 -40 -10

-120 -60 -30
Percentage of cells with SPB defects

<table>
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<tr>
<th></th>
<th>N=</th>
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<td>WT</td>
<td>116</td>
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<tr>
<td>Otrt1Δ</td>
<td>56</td>
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<tr>
<td>Internal TELO</td>
<td>72</td>
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<td>Otrt1Δ TELO plasmid</td>
<td>53</td>
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<tr>
<td>Otrt1Δ pot1Δ Internal TELO</td>
<td>45</td>
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N= 53
A

$Otrt1\Delta\ bqt1\Delta$ + Internal $TELO$  SPB Chromatin Tubulin

-100 -70 -40 -10 10 20 40 70 90 110
Prophase Meiosis I Meiosis II

B

$Otrt1\Delta\ rap1\Delta$ + Internal $TELO$  SPB Chromatin Tubulin

-70 -40 0 20 40 70 80 100 120 160
Prophase Meiosis I Meiosis II
FIGURE S5

A

Otrt1Δ  SPB Chromatin Tubulin

Prophase  Meiosis I  Meiosis II

B

Otrt1Δ rec12Δ  SPB Chromatin Tubulin

Prophase  Meiosis I  Meiosis II

C

Otrt1Δ rec12Δ  SPB Chromatin Tubulin

Prophase  Meiosis I  Meiosis II

D

Otrt1Δ rec12Δ  SPB Chromatin Tubulin

Prophase  Meiosis I  Meiosis II