Fennell et al., http://www.jcb.org/cgi/content/full/jcb.201409058/DC1

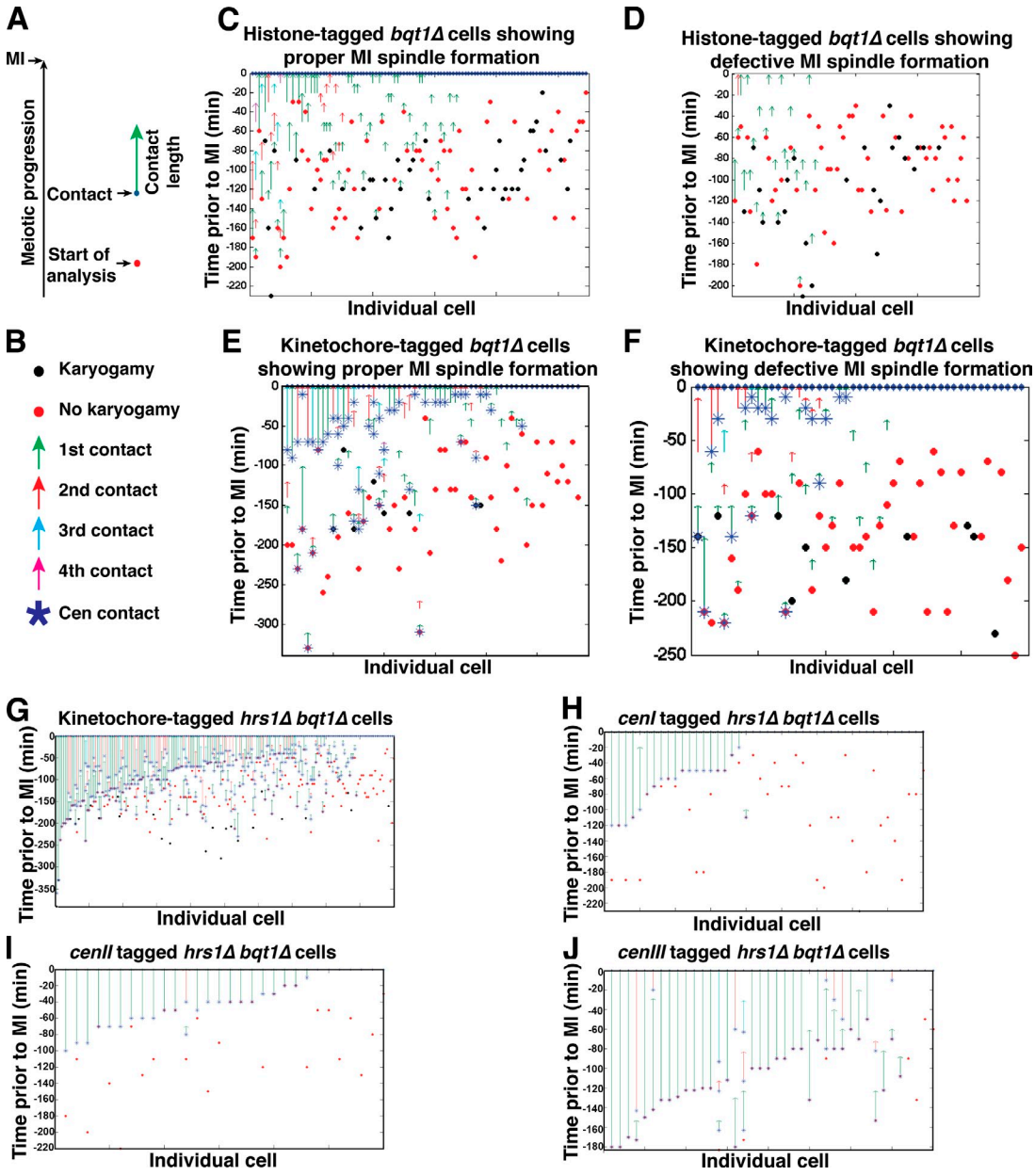


Figure S1. **Single cell analysis of chromatin/centromere–SPB contacts in *bqt1Δ* meiocytes.** All movies represented were acquired using 10-min time-lapse intervals. (A) Schematic explaining the vectors used to represent each individual cell (see Material and methods). *t* = 0 is the time point 10 min before spindle formation. Each observed chromatin–SPB contact (C and D, in strains lacking tagged centromere proteins but harboring tagged histone H3) or centromere–SPB contact (E and F, in strains harboring tagged centromeric Mis6) is represented by a vector whose length indicates the duration of the contact. Contacts are grouped according to accumulated contact duration (greater contact durations on left side of each graph). (B) Key for schematic: red dots indicate the start of analysis for zygotes in which karyogamy was not observed (i.e., zygotes that had undergone karyogamy before filming began); black dots indicate the start of analysis of zygotes in which karyogamy was observed. In E and F, asterisks indicate contact mediated by a centromere. (G–J) Vector graphs include only those cells displaying proper MI spindle formation. (G) Analyses using Mis6-GFP as a centromere marker. The faintness of this marker leads to the appearance of cells showing no apparent centromere–SPB contact. Using the brighter Swi6-GFP as a marker for centromeres, we observed contact throughout in all *hrs1Δbqt1Δ* and *dhc1Δbqt1Δ* cells (not depicted). Data in C and D, E and F, G, and H–J are represented by bar graphs in Fig. 1 D, Fig. 2 (D–G), Figs. 3 F and 4 (G–I), and Fig. 6 E, respectively.

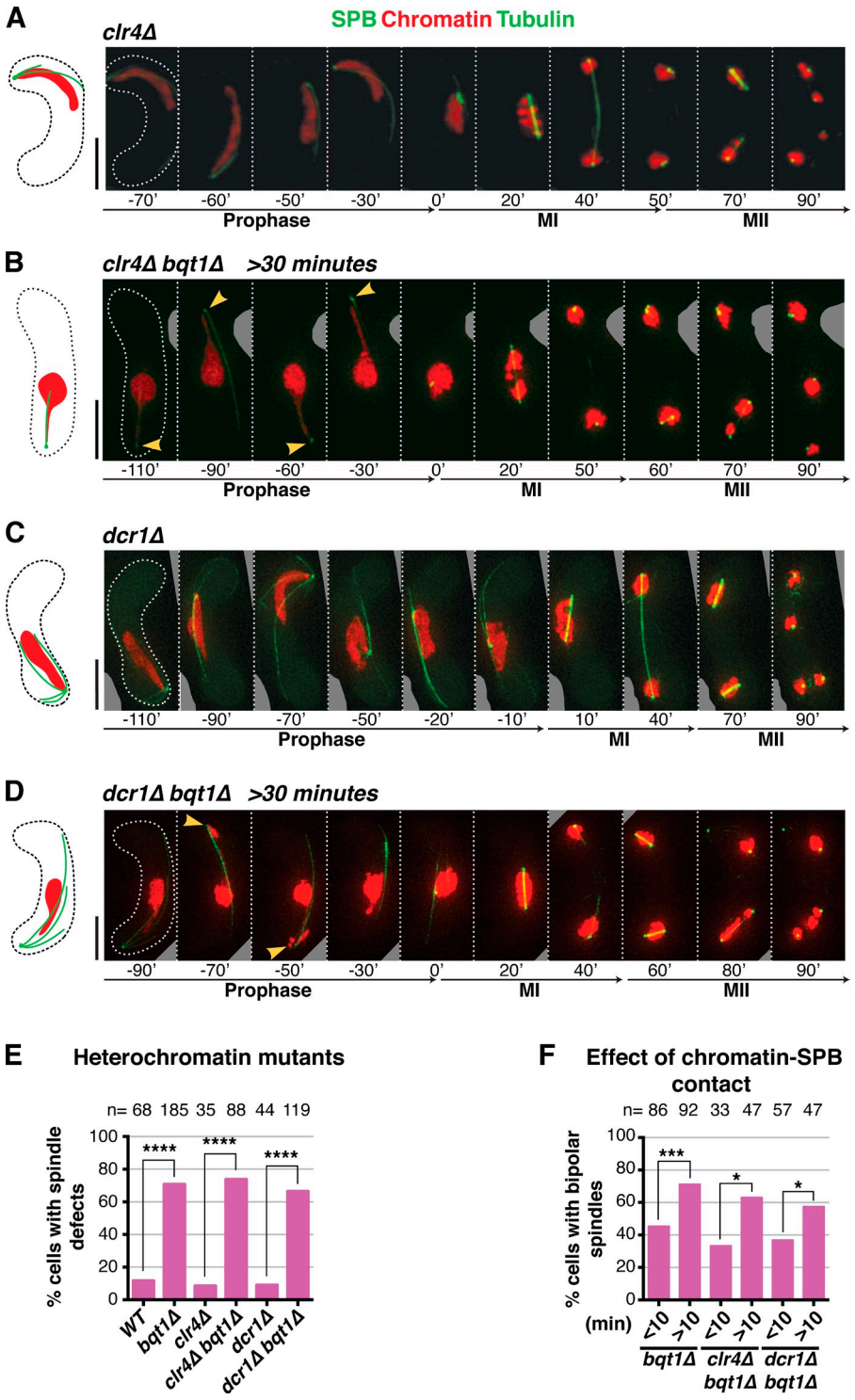


Figure S2. **Rescue of the *bqt1Δ* spindle defect by centromere–SPB contact requires neither Clr4 nor Dcr1.** (A–D) Series of frames of films of meiocytes carrying tags as in Fig. 1. Numbering as in Fig. 1. Bars, 5 µm. (A) *clr4Δ* zygotes show proper bipolar spindle formation. (B) *clr4Δ bqt1Δ* zygote showing persistent contact (indicated by yellow arrowheads) followed by bipolar spindle formation. (C) *dcr1Δ* zygote with *WT*-like spindle formation. (D) *dcr1Δ bqt1Δ* zygote showing persistent contact (indicated by yellow arrowheads) followed by bipolar spindle formation. (E) Quantitation of the overall spindle defects at MI and MII. (F) Quantitation of bipolar MI spindle formation as a function of chromatin–SPB contact. *n* is the total number of cells scored from at least two (E) and more than five (F) independent experiments. ****, $P < 0.0001$; ***, $0.0001 < P < 0.001$; *, $0.01 < P < 0.05$.

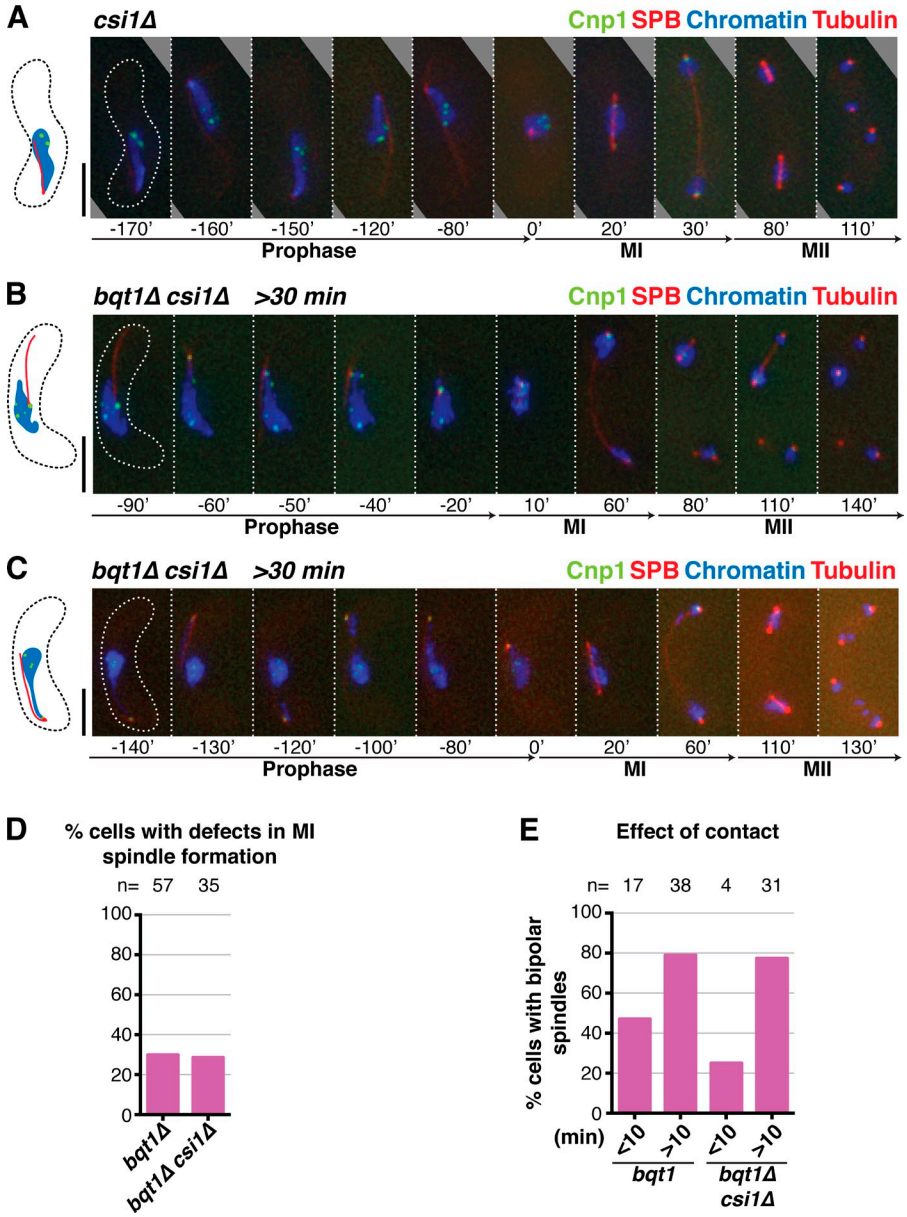


Figure S3. **Centromere–SPB interactions and spindle rescue in *bqt1Δ* meiocytes occurs independently of Csi1.** (A–C) Series of frames of films from meiocytes harboring the tags detailed in Fig. 2 H. Numbering as in Fig. 1. Bars, 5 µm. (A) *csi1Δ* zygotes show normal meiotic progression. (B and C) *bqt1Δ csi1Δ* zygotes displaying >30-min contact preceding successful bipolar spindle formation. (D) Quantitation of deficiency in MI spindle formation. (E) Quantitation of the effect of centromere–SPB contact on bipolar MI spindle formation. Contact occurs to the same extent and with the same effect with or without Csi1. *n* is the total number of cells scored from greater than three independent experiments.

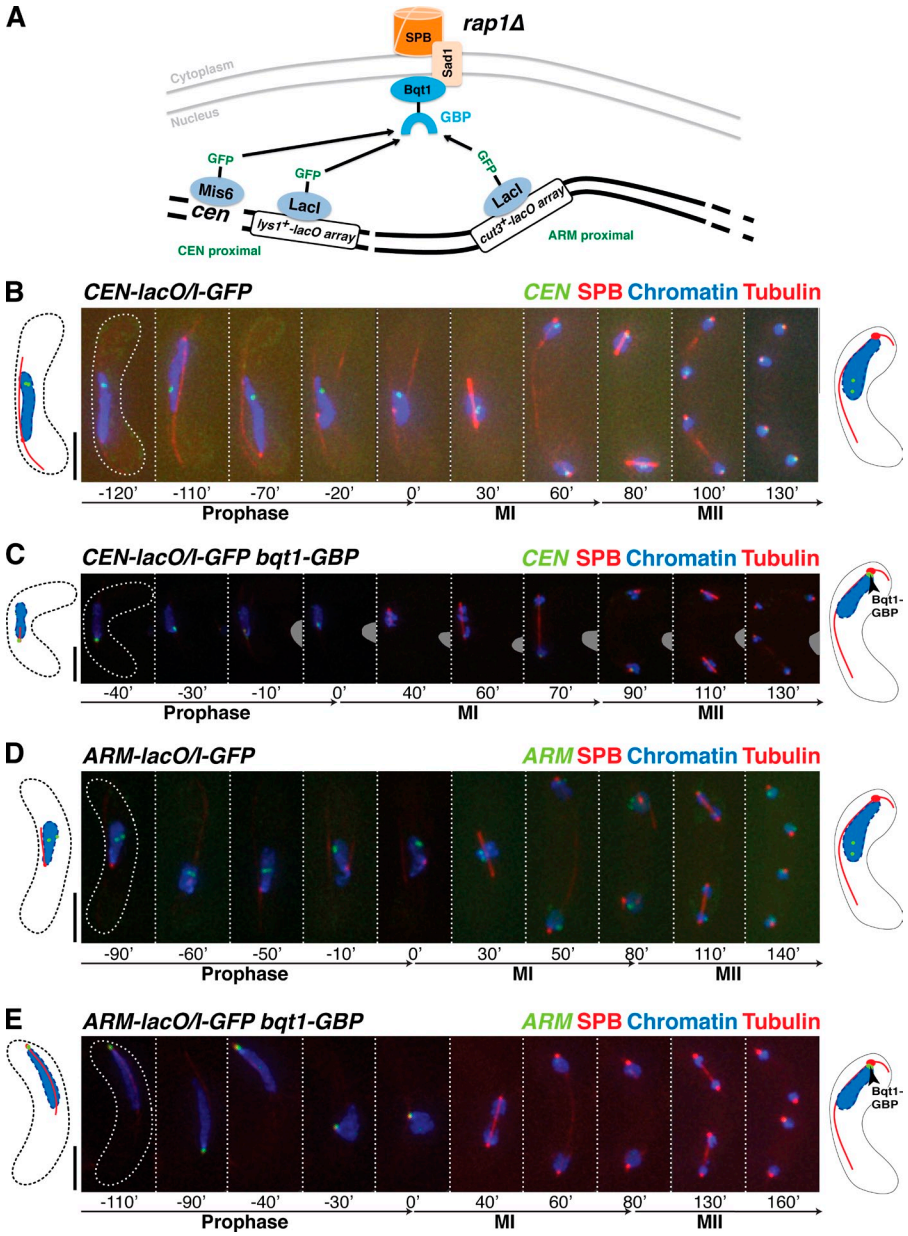


Figure S4. **Forcing SPB interactions with specific genomic loci does not affect meiotic progression in a bouquet-proficient background (controls for Fig. 7).** (A) Schematic of the GBP-GFP system used to force CEN-proximal and ARM-proximal (euchromatic) SPB interactions. (B–E) Frames of films of meiocytes harboring the specified tags: SPB, chromatin, and tubulin tagged as in Fig. 2 along with LacI-GFP; *lacO* arrays near *lys1*$^+$ and *cut3*$^+$ genes, respectively; Bqt1 is endogenously fused at its C terminus with GBP. Schematics on the right of each series show the expected prophase phenotype (NM outlined with a dashed blue line); numbering as in Fig. 1. Bars, 5 µm. (B) *lys1*$^+$*-lacO/I-GFP* meiocyte shows normal meiotic progression. (C) *lys1*$^+$*-lacO/I-GFP bqt1-GBP* meiocyte shows successful recruitment of locus to the SPB while maintaining meiotic progression. (D and E) Equivalent to B and C but with *cut3*$^+$*-lacO/I-GFP*. The data shown are from a single representative experiment out of more than three repeats.

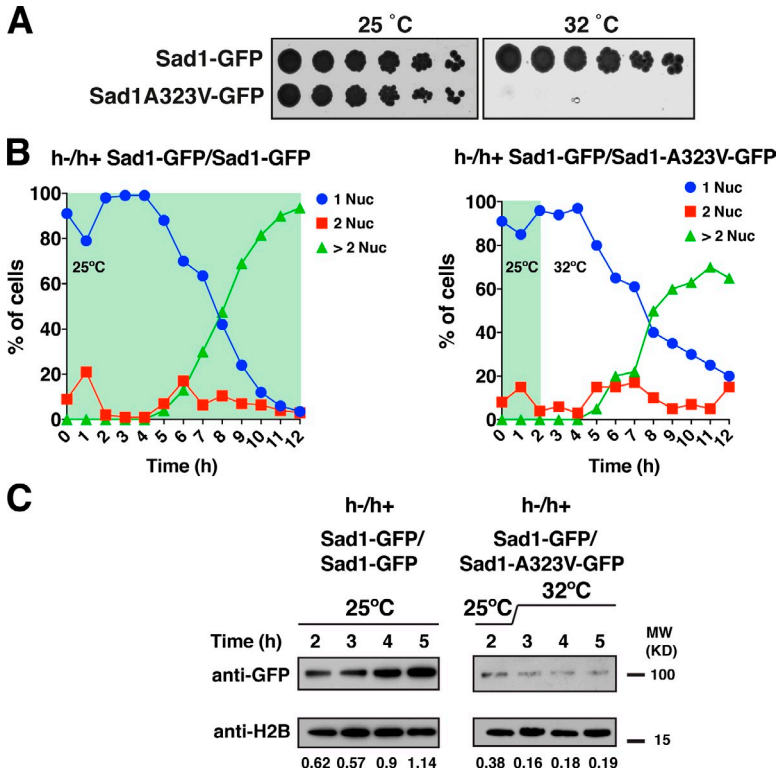


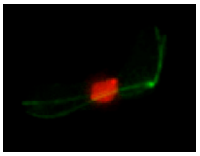
Figure S5. **Analysis of Sad1 protein levels in *sad1⁺/sad1-A323V* cells (supplement to Fig. 8).** (A) Serial dilutions (fivefold) of log-phase *WT* (Sad1-GFP) and cells harboring Sad1-A323V-GFP, grown on rich agar plates at 25 or 32°C, the nonpermissive temperature for Sad1-A323V. (B) Exponentially growing diploid cells harboring two endogenously tagged copies of Sad1-GFP or Sad1-GFP/Sad1-A323V-GFP were induced to undergo meiosis. Samples were taken every hour and stained with DAPI to assess meiotic progression; the percentage with indicated numbers of nuclei per cell (1 nucleus indicating prophase, 2 indicating MI, and >2 indicating MII) is shown for 200 cells at each time point. After 2 h of meiotic induction at 25°C, when the majority of cells had started prophase, the temperature was switched to 32°C. (C) Western blot analysis of samples from the experiments shown in B. Labels to the left indicate the antibodies used to probe the blots. Numbers below each row indicate relative Sad1-GFP intensity of each sample compared with the loading control (histone H2B). The data shown are from a single representative experiment out of three repeats.

Table S1. **Strains constructed and analyzed in this work**

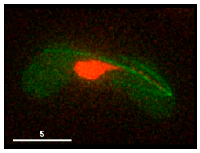
JCF number	Mating type	Genotype	Use	Video	Origin
7154	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6*	Figs. 1, S1, and S2		Lab stock
7158	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6 bqt1::hygMX6*	Figs. 1, S1, and S2	1 and 2	Lab stock
7161	h^{+}/h^{-}	*ade6-M210/ade6-M216 leu1-32/leu1-32 lys1:Pnmt1:GFP-Atb2/lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+/Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6/Sid4-GFP:kanMX6*	Fig. 1		Lab stock
7163	h^{+}/h^{-}	*ade6-M210/ade6-M216 leu1-32/leu1-32 lys1:Pnmt1:GFP-Atb2/lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+/Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6/Sid4-GFP:kanMX6 bqt1::hygMX6/bqt1::hygMX6*	Fig. 1		Lab stock
8214	h^{+}/h^{-}	*ade6-M216/ade6-M210 Hht1-CFP:his3/Hht1-CFP:his3 taz1-mCherry:natMX6/taz1-mCherry:natMX6 sad1-GFP:kanMX6/sad1.A323V-GFP:kanMX6*	Fig. S5		This study
8297	h^{90}	*leu1-32 Sad1-RFP:leu1+ bqt1-GBP:HygMX6 rap1::natMX6 mis6-GFP:kanMX6*	Fig. 8		This study
9471	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6 clr4::natMX6*	Fig. S2		This study
9474	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6 bqt1::hygMX6 clr4::natMX6*	Fig. S2		This study
9984	h^{+}/h^{-}	*ade6-M210/ade6-M216 Pnda3-mCherry-Atb2:aur1/Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6/Sid4-mCherry:natMX6 Mis6-GFP:kanMX6/Mis6-GFP:kanMX6*	N/A		This study
9988	h^{+}/h^{-}	*ade6-M210/ade6-M216 Pnda3-mCherry-Atb2:aur1/Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6/Sid4-mCherry:natMX6 Mis6-GFP:kanMX6/Mis6-GFP:kanMX6 bqt1::hygMX6/bqt1::hygMX6*	N/A		This study
10100	h^{90}	*ade6-M210 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6*	Figs. 2, S1, and S3		This study
10128	h^{90}	*ade6-M210 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 bqt1::hygMX6*	Figs. 2, S1, and S3	3	This study
10193	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6 dcr1::natMX6*	Fig. S2		This study
10196	h^{90}	*ade6-M210 leu1-32 lys1:Pnmt1:GFP-Atb2 Hht1-mRFP:kanMX6::leu1+ Sid4-GFP:kanMX6 bqt1::hygMX6 dcr1::natMX6*	Fig. S2		This study
10263	h^{90}	*ade6-M216 leu1-32 ura4-D18 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 lys+:Cnp1-GFP bqt1::hygMX6*	Fig. 2		This study
10322	h^{90}	*his3-D1 Hht1-CFP::his3+MX6 Sid4-mRFP::kanMX6:leu1+ Hrs1-GFP2xFLAG:kanMX6 Pnda3-mCherry-Atb2:aur1*	Fig. 5		This study
10323	h^{90}	*his3-D1 Hht1-CFP::his3+MX6 Sid4-mRFP::kanMX6:leu1+ Hrs1-GFP2xFLAG:kanMX6 Pnda3-mCherry-Atb2:aur1 bqt1::hygMX6*	Fig. 5		This study
10352	h^{90}	*ade6-M210 leu1-32 ura4-D18 Hht1-CFP:kanMX6 Sad1-GFP:hygMX6 Sid4-mCherry:natMX6*	Fig. 8		This study
10355	h^{90}	*ade6-M210 leu1-32 ura4-D18 Hht1-CFP:kanMX6 Sad1-GFP:hygMX6 Sid4-mCherry:natMX6 bqt1::LEU2*	Fig. 8		This study
10361	h^{90}	*ade6-M210 his3-D1 leu1-32 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 hrs1::hygMX6*	Figs. 4 and S1		This study
10373	h^{90}	*ade6-M210 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 hrs1::hygMX6 bqt1::hygMX6*	Figs. 4 and S1		This study
10375	h^{90}	*ade6-M210 his3-D1 leu1-32 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6*	Fig. 4		This study
10376	h^{90}	*ade6-M210 his3-D1 leu1-32 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 dhc1::hygMX6 bqt1::hygMX6*	Fig. 4	5	This study
10377	h^{90}	*ade6-M210 his3-D1 leu1-32 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 bqt1::hygMX6*	Fig. 4		This study
10378	h^{90}	*ade6-M210 his3-D1 leu1-32 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 Mis6-GFP:kanMX6 dhc1::hygMX6*	Fig. 4		This study
10389	h^{90}	*ade6-M210 leu1-32 ura4 his7+::lacI-GFP lys1+::lacO Sad1-CFP:kanMX6 bqt1::LEU2 hrs1::hygMX6*	Figs. 6 and S1		This study
10392	h^{90}	*leu1-32 z:natR-Padh31-tetR-tomato cnt2:tetO*2:ura4 Sad1-CFP:kanMX6 bqt1::LEU2 hrs1::hygMX6*	Figs. 6 and S1		This study
10396	h^{+}	*wee1.50*	N/A		Nurse lab
10397	h^{-}	*wee1.50*	N/A		Nurse lab
10398	h^{+}	*cdc25.22 (C532Y)*	N/A		Nurse lab
10399	h^{-}	*cdc25.22 (C532Y)*	N/A		Nurse lab

Table S1. **Strains constructed and analyzed in this work** *(Continued)*

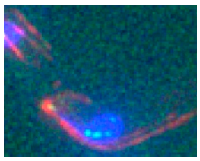
JCF number	Mating type	Genotype	Use	Video	Origin
10408	h^-	*wee1.50 bqt1::hygMX6*	N/A		This study
10409	h^+	*wee1.50 bqt1::hygMX6*	N/A		This study
10411	h^-	*cdc25.22 (C532Y) bqt1::hygMX6*	N/A		This study
10421	h^+	*cdc25.22 (C532Y) bqt1::hygMX6*	N/A		This study
10423	h^-/h^+	*ade6-M216/ade6-M210 Hht1-CFP:his3/Hht1-CFP:his3 taz1-mCherry:natMX6/taz1-mCherry:natMX6 sad1-GFP:kanMX6/sad1-GFP:kanMX6*	Fig. S5		This study
10439	h^{90}	*leu1-32 lys1-131 ura4D-18 ade6 [::ura4+-kanMX6-lacO] his7+::lacI-GFP Sad1-mRFP:hygMX6 bqt1::LEU2 hrs1::natMX6*	Figs. 6 and S1		This study
10466	h^{90}	*leu1-32 Hht1-CFP:hisMX6 Sid4-mCherry:natMX6 Hrs1-GFP-2xFLAG:kanMX6 Pnda3-mCherry-Atb2:aur1 pLT1-2(nmt1:lsh1-GFP-LEU2)*	Fig. 5		This study
10467	h^{90}	*leu1-32 Hht1-CFP:hisMX6 Sid4-mCherry:natMX6 Hrs1-GFP-2xFLAG:kanMX6 Pnda3-mCherry-Atb2:aur1 bqt1::hygMX6 pLT1-2(nmt1:lsh1-GFP-LEU2)*	Fig. 5		This study
11629	h^{90}	*ade6-M216 leu1-32 ura4-D18 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 lys+:Cnp1-GFP csi1::kanMX6*	Fig. S3		This study
11631	h^{90}	*ade6-M216 leu1-32 ura4-D18 his3-D1 Hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Sid4-mCherry:natMX6 lys+:Cnp1-GFP bqt1::hygMX6 csi1::kanMX6*	Fig. S3		This study
11635	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 his7+::LacI-GFP lys1+::LacO lig4::kanMX6 Bqt1-GBP:hygMX6*	Fig. S4		This study
11636	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-atb2:aur1 Hht1-CFP:his3 his7+::LacI-GFP lys1+::LacO lig4::kanMX6*	Fig. S4		This study
11639	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 his7+::LacI-GFP lys1+::LacO lig4::kanMX6 rap1::ura4 Bqt1-GBP:hygMX6*	Fig. 7		This study
11640	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 his7+::LacI-GFP lys1+::LacO lig4::kanMX6 rap1::ura4*	Fig. 7		This study
11646	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 cut3+::LacO his7+::LacI-GFP lig4::kanMX6*	Fig. S4		This study
11648	h^{90}	*leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 cut3+::LacO his7+::LacI-GFP lig4::kanMX6 Bqt1-GBP:hygMX6*	Fig. S4		This study
11651	h^{90}	*ade6 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 cut3+::LacO his7+::LacI-GFP lig4::kanMX6 rap1::ura4 Bqt1-GBP:hygMX6*	Fig. 7		This study
11653	h^{90}	*leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 Hht1-CFP:his3 cut3+::LacO his7+::LacI-GFP lig4::kanMX6 rap1::ura4*	Fig. 7		This study
11715	h^{90}	*ade6-M216 his3-D1 leu1-32 ura4-D18 Pnda3-mCherry-Atb2:aur1 Mis6-GFP:kanMX6 Sid4-mCherry:natMX6 rap1::ura4 lig4::kanMX6 Hht1-CFP:his3*	Fig. 3		This study
11733	h^{90}	*ade6-M216 his3-D1 leu1-32 ura4-D18 Pnda3-mCherry-Atb2:aur1 Mis6-GFP:kanMX6 Sid4-mCherry:natMX6 rap1::ura4 lig4::kanMX6 Hht1-CFP:his3 Bqt1-GBP:hygMX6*	Fig. 3	4	This study
11751	h^{90}	*ade6-M216 leu1-32 his3-D1 ura4-D18 Sid4-mCherry:natMX6 Pnda3-mCherry-Atb2:aur1 rap1::ura4 lig4::kanMX6 Bqt1-GBP:hygMX6*	Fig. 3		This study
12532	h^{90}	*leu1 Sad1-RFP:LEU1 bqt1-GBP-HygMX6 rap1::natMX6*	Fig. 8		This study



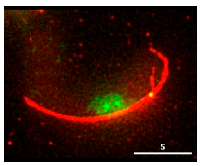
Video 1. ***bqt1Δ* cells with no contact show defective spindle formation.** A *bqt1Δ* meiocyte harboring GFP-tagged tubulin and SPB and mRFP-tagged histone H3 (*h*^{90} *Atb2-GFP Sid4-GFP Hht1-mRFP bqt1Δ*) was imaged by time-lapse microscopy at 27°C in an Environmental Chamber with a DeltaVision Spectris (Applied Precision) comprising a widefield inverted epifluorescence microscope (IX70; Olympus), a UPlanSapo 100× NA 1.4 oil immersion objective (Olympus), and a charge coupled device CoolSnap HQ camera (Photometrics). Images were acquired over 26 focal planes at a 0.35-µm step size with frames taken every 10 min for 7 h.



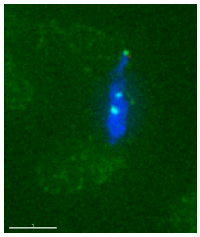
Video 2. ***bqt1Δ* cells with persistent (>30 min) chromatin–SPB contact show bipolar spindle formation.** A *bqt1Δ* meiocyte (*h*^{90} *Atb2-GFP Sid4-GFP Hht1-mRFP bqt1Δ*) was imaged by time-lapse microscopy at 27°C in an Environmental Chamber with a DeltaVision Spectris (Applied Precision) using the same settings as in Video 1.



Video 3. **In the majority of cases, chromatin–SPB contact is mediated by the centromere.** A *bqt1Δ* meiocyte (*h*^{90} *Atb2-mCherry Sid4-mCherry Hht1-CFP Mis6-GFP bqt1Δ*) was imaged by time-lapse microscopy at 27°C in an Environmental Chamber with a DeltaVision Spectris (Applied Precision) as described in Video 1.



Video 4. **Artificial maintenance of centromere–SPB contacts ensures bipolar spindle formation.** A *rap1Δ lig4Δ* meiocyte (*h*^{90} *Atb2-mCherry Sid4-mCherry Hht1-CFP Mis6-GFP rap1Δ lig4Δ Bqt1-GBP*) was imaged by time-lapse microscopy at 27°C as described in Video 1.



Video 5. **Loss of nuclear movement results in centromere–SPB contact throughout and bipolar spindle formation.** A *bqt1Δ dhc1Δ* meiocyte (*h*^{90} *Atb2-mCherry Sid4-mCherry Hht1-CFP Mis6-GFP bqt1Δ dhc1Δ*) was imaged by time-lapse microscopy. The microscope settings used are described in Video 1.

A MATLAB script for the vector diagram of the range of contact lengths in individual cells shown in Fig. S1 is provided online.