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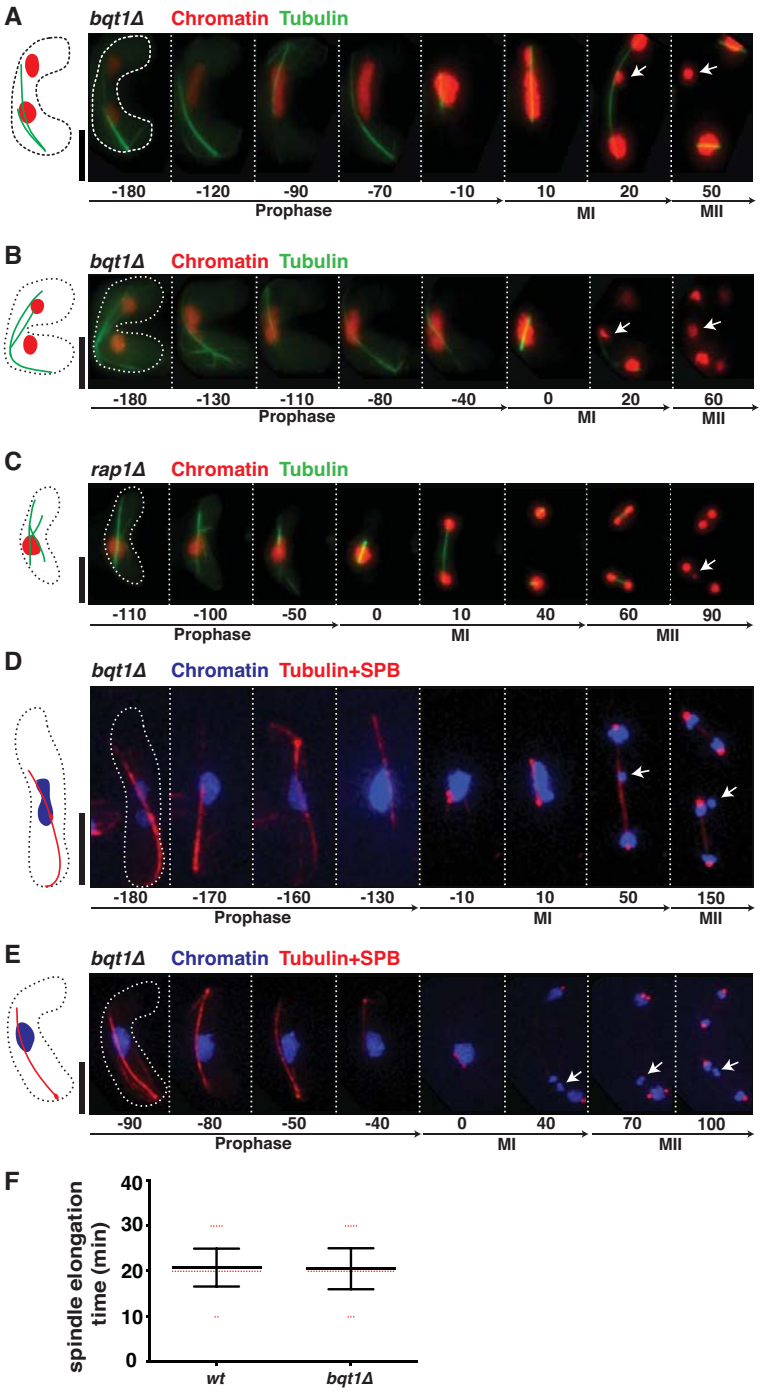
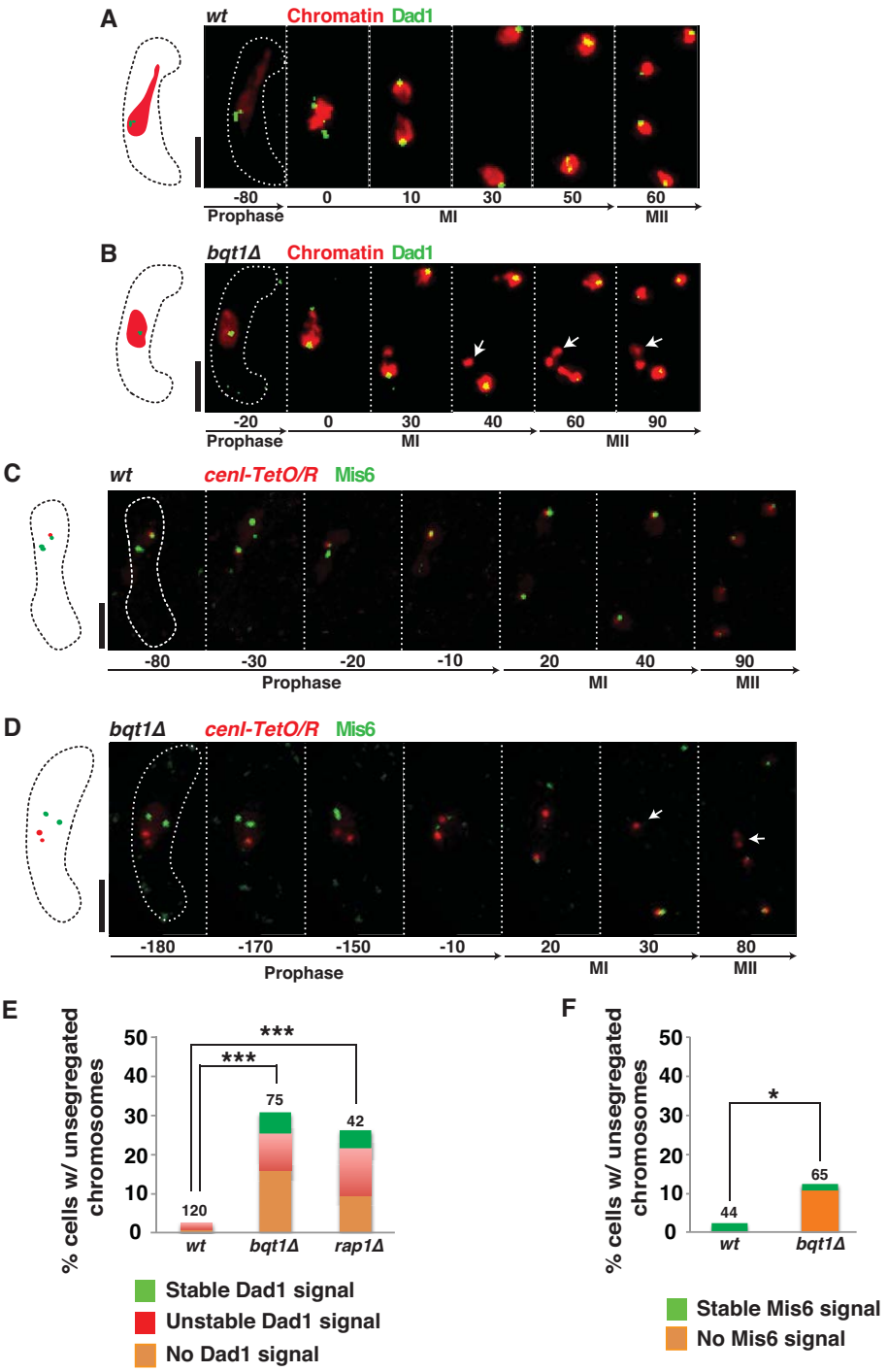
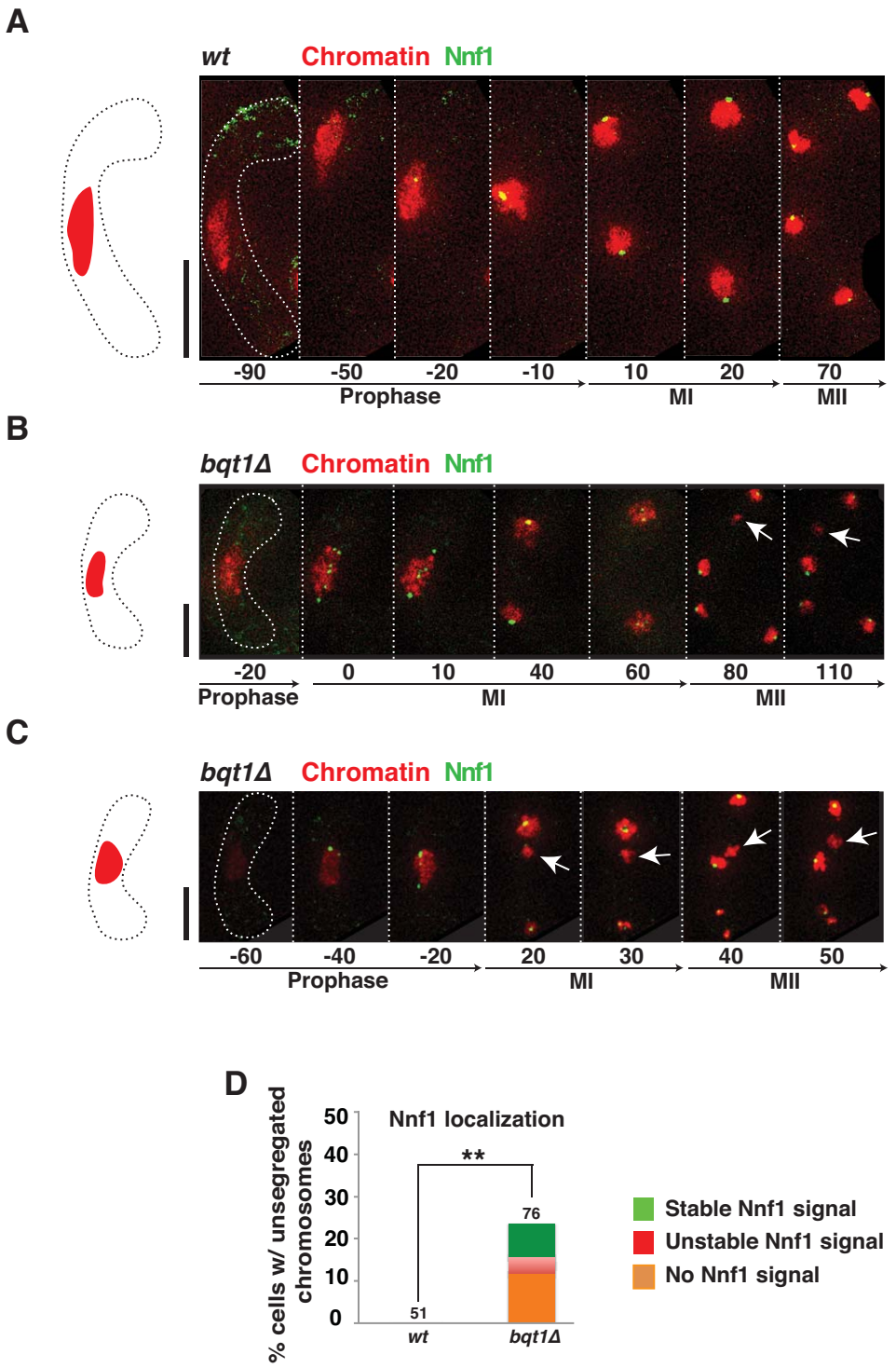


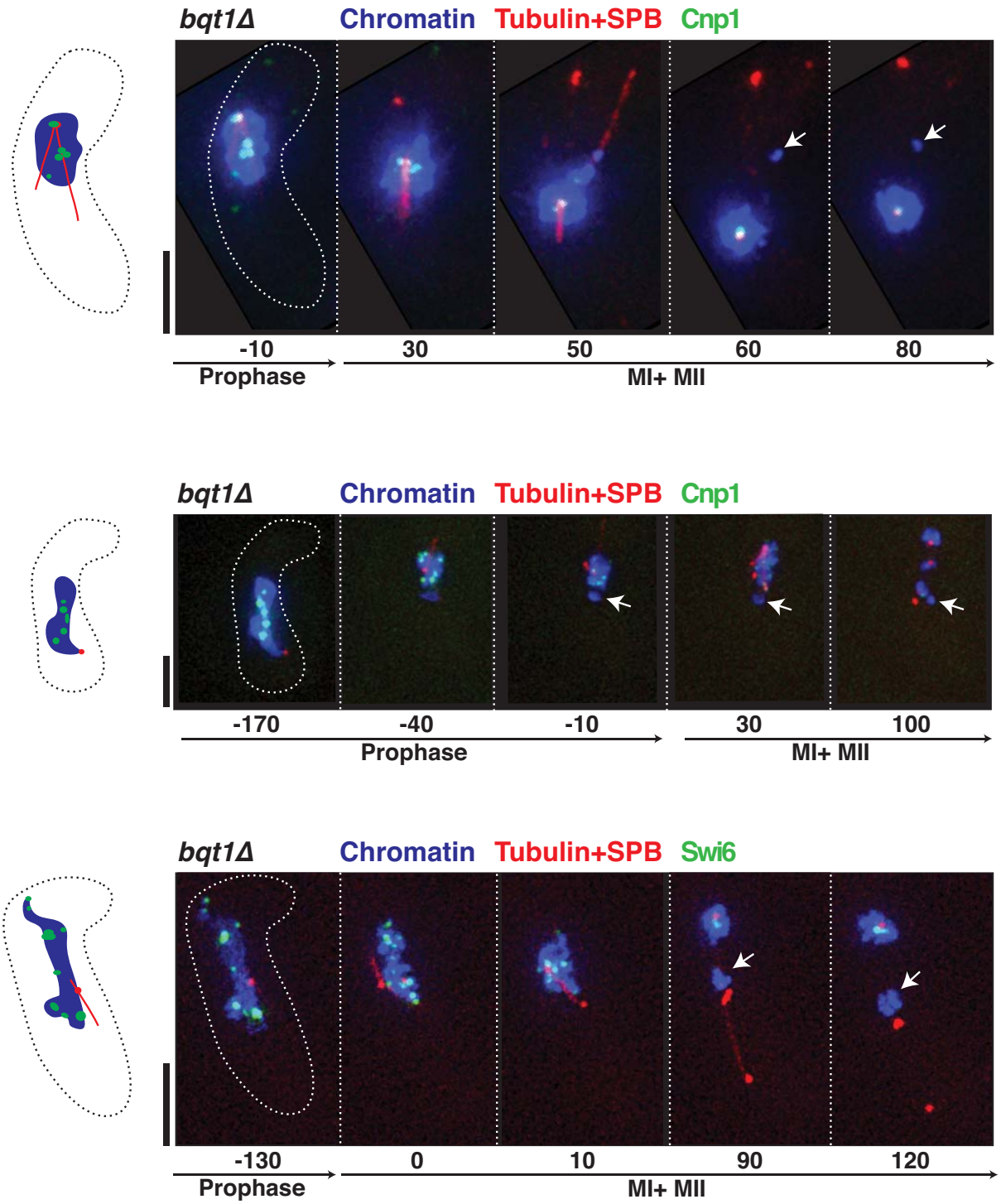
Supplementary Figure 1 Bouquet deficient meiocytes show failure of chromosome attachment to correctly formed spindles. (A-C) Examples of bouquet-deficient cells undergoing meiosis. Tubulin and histone H3 are observed *via* ectopically expressed GFP-Atb2 (green) and endogenous mRFP tagging of one of the two alleles encoding Hht1 (red), respectively. Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5μm. (D-E) Tubulin and histone H3 are observed *via* endogenously tagged Atb2-mRFP (red) and endogenous CFP tagging of one of the two alleles encoding Hht1 (blue), respectively. Labels as in (A). (A-B, D-E) *bqt1Δ* meiosis. The spindle forms correctly but some chromosomes (arrows) fail to attach to those spindles and remain unsegregated (C) *rap1Δ* meiosis. The spindle forms correctly but some chromosomes (arrows) fail to attach to those spindles and remain unsegregated.(F) Bouquet deficient cells form spindles with normal elongation rates. The time from anaphase I onset to the moment of maximal spindle length was measured. Red dots represent values for each individual cell; black lines represent the mean +/- standard error. Wt and *bqt1Δ* cells with good spindles have identical maximal spindle lengths and rates of spindle elongation.



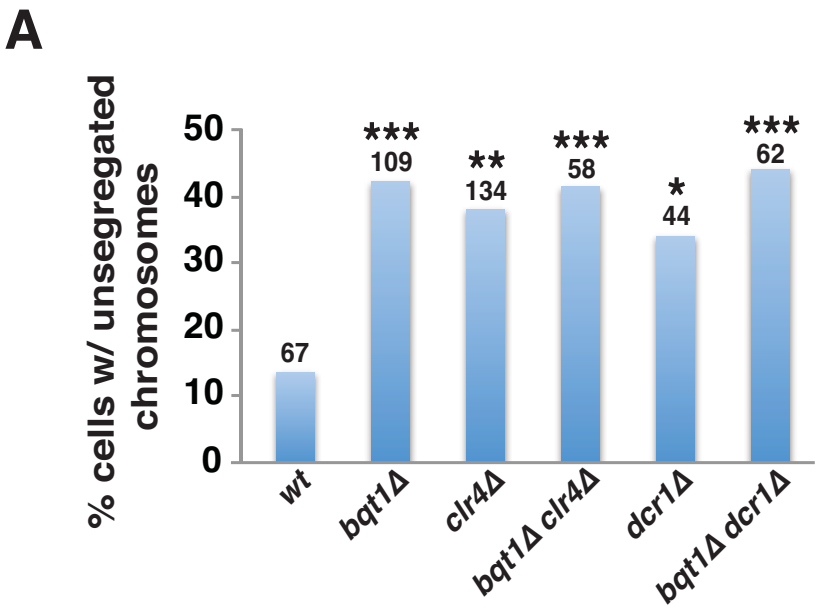
Supplementary Figure 2 Bouquet deficient meiocytes show incomplete recruitment of Dad1 and Mis6 to centromeres. (A-B) Series of frames from films of cells undergoing meiosis. Dad1 is observed *via* endogenous GFP tagging, and histone H3 *via* mRFP tagging as in Fig. 1. Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5μm. (A) wt meiosis. Dad1-GFP appears at all chromatin masses at MI and MII. (B) *bqt1Δ* meiosis. Some chromosomes (arrows) fail to recruit Dad1 and remain unsegregated. (C-D) Series of frames from films of cells undergoing meiosis. Mis6 is observed via endogenous functional GFP tagging. *cenI-TetO/R* is observed as in Fig. 2 Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5μm. (C) wt meiosis. Mis6-GFP is correctly recruited to centromere I. (D) *bqt1Δ* meiosis. In some cases centromere I fails to recruit Mis6 and remains unsegregated. (E) Quantitation of Dad1 localization. For each genetic background, the percentage of cells harbouring unsegregated chromosomes is plotted; the superimposed colour code specifies the pattern of Dad1-GFP signal in those cells. See Methods for definitions of 'stable' and 'unstable'. Number of cells filmed is indicated above each lane. Asterisks indicate significant difference from wt calculated using Fisher's exact test (wt-*bqt1Δ* $p=10^{-8}$, wt-*rap1Δ* $p=2*10^{-5}$; see Methods). (F) Quantitation of Mis6 localization on centromere I. The percentage of cells harbouring unsegregated centromere I is plotted; the superimposed colour code specifies the pattern of Mis6-GFP signal on those centromeres. Number of cells filmed is indicated above each lane. Asterisk indicates significant difference from wt calculated using Fisher's exact test (wt- *bqt1Δ* p=0.04).

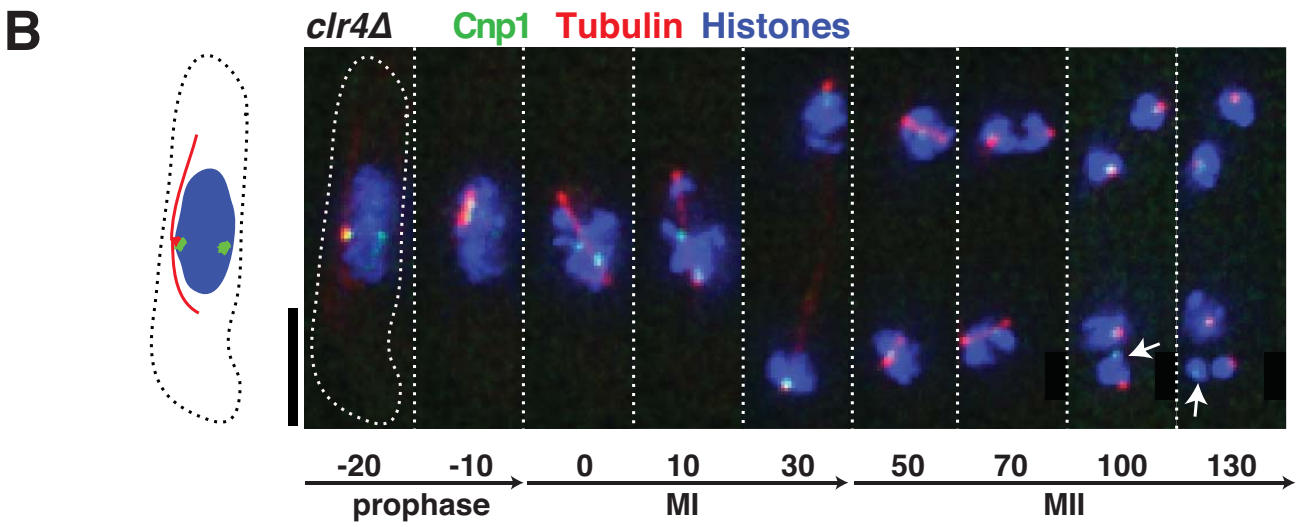


Supplementary Figure 3 Bouquet deficient meiocytes fail to properly assemble outer kinetochores. (A-C) Nnf1 and chromosomes are observed *via* endogenously tagged functional Nnf1-GFP and Hht1-mRFP (as in Fig. 1), respectively. Nnf1 disappears from centromeres in early prophase and relocalizes to centromeres 10-40 minutes before metaphase I. Labels as in Supplementary figure 1. (A) wt meiosis. Nnf1-GFP appears on all chromatin masses at MI and MII. (B-C) *bqt1Δ* meiosis. Some chromosomes fail to recruit Nnf1 and remain unsegregated (arrows). (D) Quantitation of Nnf1 localization. For each genetic background, the percentage of cells harbouring unsegregated chromosomes is plotted; the superimposed colour code specifies the pattern of Nnf1-GFP signal in those cells. See Methods for definitions of 'stable' and 'unstable'. Labels as in Supplementary figure 1. (wt-*bqt1Δ* p=0.0014).

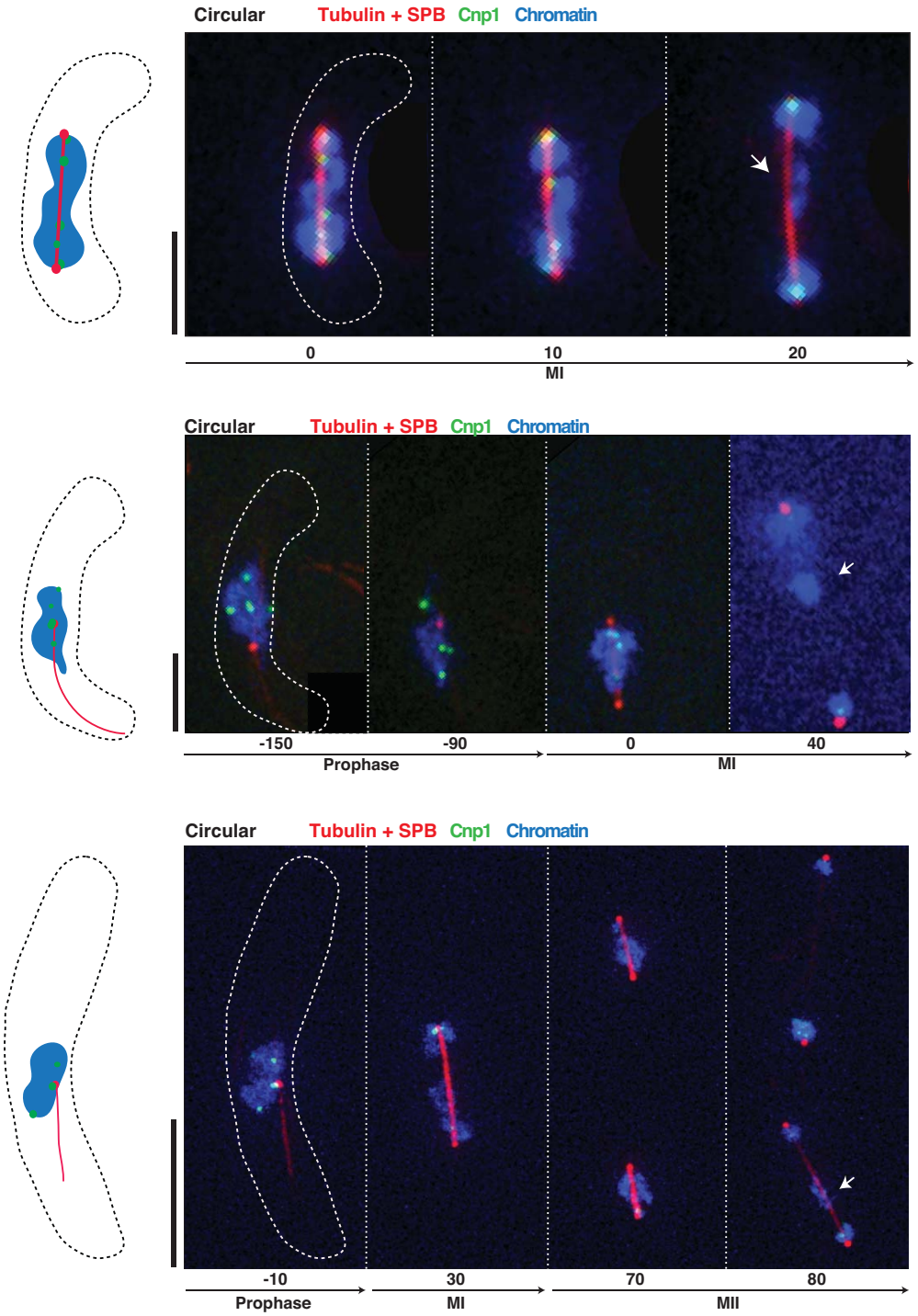


Supplementary Figure 4 Centromere assembly failure occurs not only in meiocytes with functional spindles, but also in those with dysfunctional spindles. Series of frames from films of cells undergoing meiosis. Cnp1 and Swi6 are observed as in Fig. 2. Histone H3 is observed by CFP tagging as in Supplementary Fig. 1, and tubulin and SPB by endogenous mCherry tagging of Atb2 and Sid4. Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5μm. In these three examples, SPBs are scattered far from chromatin and spindles fail to assemble properly. Some chromosomes lacking kinetochore signals (either Cnp1 or Swi6; arrows) can be discerned as they separate from the main chromatin mass.

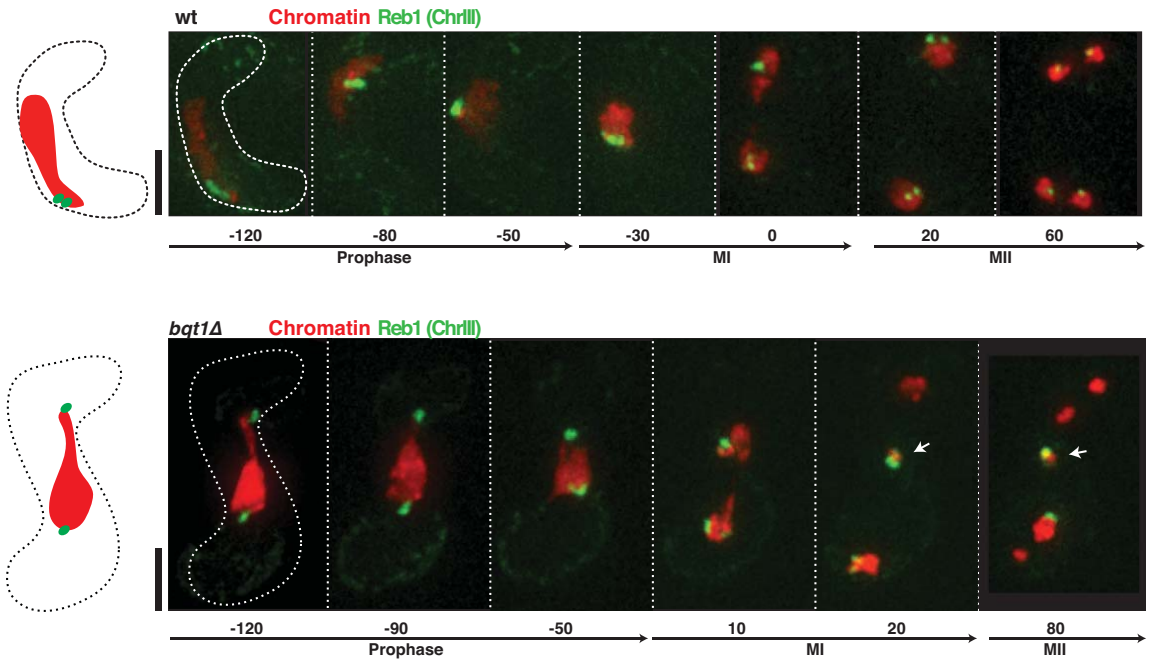




Supplementary Figure 5 Meiocytes deficient in pericentromeric heterochromatin formation show centromere assembly defects. (A) Genetic epistasis analysis of mutations that abolish the bouquet or compromise heterochromatin assembly. Cumulative frequencies of non-attachment events in MI and MII are observed *via* GFP-Atb2, Sid4-GFP and Hht1-mRFP (as in Fig 1). Asterisks indicate that all mutant backgrounds differ significantly from wt; no significant difference is observed among the various mutant genotypes. Number of cells filmed is indicated above each bar. Significance was calculated using Fisher's exact test (wt-*bqt1Δ* $p=5*10^{-5}$, wt-*clr4Δ* p=0.002, wt-*clr4Δ bqt1Δ* p=0.0005, wt- *dcr1Δ* p=0.01, wt-*dcr1Δ bqt1Δ* p=0.0001, see Methods for details). (B) Series of frames of a film of a cell undergoing meiosis. Cnp1, tubulin and chromatin are observed *via* ectopically expressed Cnp1-GFP (green) atb2-mCherry (red) and Hht1-CFP (blue), respectively. Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5µm. This film shows an example of *clr4Δ* meiosis. Some chromosomes (arrows) fail to recruit cohesin and thus missegregate, while maintaining high levels of Cnp1.



Supplementary Figure 6 Impaired kinetochore function in meiocytes harbouring circular chromosomes. Series of frames of cells undergoing meiosis. Cnp1, tubulin, SPB and chromatin are observed *via* ectopically expressed Cnp1-GFP, Atb2-mCherry, Sid4-mCherry (red) and Hht1-CFP, respectively. Numbers below frames represent minutes before or after metaphase I. Scale bars represent 5μm. Circular chromosome-containing meiocytes suffer similar phenotypes to bouquet-deficient linear chromosome-containing meiocytes. In these examples, spindles form properly but some chromosomes (arrows) lack kinetochore signals and fail to attach. The top two series show MI missegregation, and the bottom shows an unsegregated chromatid at MII.



Supplementary figure 7 Chr III is not inherently protected from centromere assembly defects. A potential alternative explanation for the attachment of Chr III to the spindle in a 'circular + internal telo' background could stem from the unique presence on Chr III of the rDNA repeats, whose heterochromatic nature could conceivably protect the centromere of Chr III from effects of bouquet deficiency. To assess this possibility, we monitored the attachment of Chr III to the spindle in linear chromosome-containing *bqt1*Δ meiocytes *via* endogenously GFP-tagged and functional Reb1, a rDNA binding protein. Of 29 cells with functional spindles, 10 had chromosomes remaining in the center of the cell at anaphase I or II. In 4 of these 10, the unsegregated chromosome harboured Reb1 signal, indicating that the centromere of Chr III does not enjoy singular protection from non-attachment; rather, the internal telomere stretch affords proper kinetochore assembly on circular Chr III. Representative films are shown. Numbers below frames represent time relative to metaphase I. Scale bar represents 5μm. Chr III resides in the middle of the cell at anaphase I (arrow), indicating failed segregation.

Supplementary video legends

Supplementary video 1
Correct attachment of chromosomes to the spindle in wt meiosis. Film of a wt cell undergoing meiosis. Tubulin (green) and histone H3 (red) are observed as in Fig. 1. All chromosomes attach correctly to the spindle and segregate correctly in MI and MII.

Supplementary video 2
Bouquet deficient meiocytes fail in chromosome attachment to correctly formed spindles. Film of a *rap1Δ* cell undergoing meiosis. Tubulin (green) and histone H3 (red) are observed as in Fig. 1. Bouquet formation fails, and a chromatin mass fails to associate with the spindle and remains unsegregated in MI and MII.

Supplementary videos 3-4
Nuclear rupture does not occur in bouquet deficient meiocytes. Films of *bqt1Δ* cells undergoing meiosis. Ish1-GFP (a nuclear membrane marker, green), tubulin and SPB (red) and histone H3 (blue) are observed. The nucleus remains as one entity and does not undergo breakage; nonetheless, a chromatin mass fails to associate with the spindle and remains unsegregated at MI and MII.

Supplementary video 5
Correct kinetochore assembly in wt meiosis. Film of a wt cell undergoing meiosis. Cnp1 (green) and histone H3 (red) are observed as in Fig. 2. Cnp1-GFP appears at all chromatin masses at MI and MII.

Supplementary video 6
Bouquet deficient meiocytes fail to properly load Cnp1. Film of a *bqt1Δ* cell undergoing meiosis. Cnp1 (green) and histones (red) are observed as in Fig. 2. Note that at MI a chromatin mass remains unsegregated. This unsegregated chromatin mass lacks detectable Cnp1-GFP.

Supplementary video 7
Correct pericentromeric heterochromatin formation in wt meiosis. Film of a wt cell undergoing meiosis. Swi6 (green) and *cenI* (red) are observed as in Fig. 3. Swi6-GFP localizes to centromeres and telomeres; the latter are seen moving with the leading edge of the nucleus (where the SPB is located) during the horsetail period. Swi6 is correctly recruited to *cenI* throughout meiosis.

Supplementary video 8
Bouquet deficient meiocytes show defects in pericentromeric heterochromatin formation. Film of a *bqt1Δ* cell undergoing meiosis. Swi6 (green) and *cenI* (red) are observed as in Fig. 3. Note that *cenI* fails to attach to the spindle and remains unsegregated. This *cenI* lacks detectable Swi6-GFP signal.

Supplementary video 9
Heterochromatin deficient meiocytes fail to properly assemble kinetochores. Film of a *clr4Δ* cell undergoing meiosis. Cnp1 (green), tubulin (red) and histone H3 (blue) are observed. Despite proper bouquet formation, a chromatin mass fails to recruit Cnp1 and remains unsegregated at MI.

Supplementary video 10
Heterochromatin deficient meiocytes show failure of chromosome attachment to correctly formed spindles. Film of a *clr4Δ* cell undergoing meiosis. Tubulin (green) and histone H3 (red) are observed. Despite proper bouquet formation, a chromatin mass fails to associate with the spindle and remains unsegregated at MI.

Supplementary video 11
Telomeres and centromeres co-localize at the onset of meiotic prophase. Film of wt azygotic meiotic prophase. Centromeres (green) and telomeres (red) are observed as in Fig. 5. A subset of centromeres and telomeres co- localize in many frames, until all telomeres localize to the SPB and all centromeres are released upon the onset of horsetail nuclear movements.

Supplementary video 12
Chromosomes associated with the bouquet are protected from centromere assembly defects. Film of a 'circular + internal telo' cell undergoing meiosis. Tubulin and SPB (red), Taz1 (green) and histone H3 (blue) are observed as in Fig. 5.. Note that the chromosome attached to spindle harbors a Taz1-YFP signal, while chromosomes off the spindle lack this signal.

Supplementary video 13
Centromeres tethered to the bouquet during prophase are protected from centromere assembly defects. Film of two 'circular + internal telo' meiocytes harboring Bqt1-GBP, *cenI-lacO/I* (green) and histone H3 (red) as in Fig. 6. In both cells, all *cenI*-containing chromosomes attach to the spindle. Chromatin collapse immediately following spindle dissolution at MI and MII is typical in 'circular' strains due to catenation of their chromosomes.