Cell

EXTENDED EXPERIMENTAL PROCEDURES

Fly Stocks and Crosses

The *grp* mutation was previously described in (Sullivan et al., 1993). GFP-Cid (Schuh et al., 2007), GFP-Mad2, GFP-BubR1 (Buffin et al., 2005), GFP-H2Av (Clarkson and Saint, 1999) and GFP-Polo (Moutinho-Santos et al., 1999) were previously described. The RFP-H2Av stock was obtained from the Bloomington stock center.

w^{1118}; p[w^+, GFP-H2Av], 70I-CreI, Sb/ TM6, Hu, Tb stock was used in Figures 2A and 2B (non-heat-shocked larvae were used as controls). y^1, w^{1118}, p[w^+, GFP-BubR1]; p[w^+; RFP-H2Av]; 70I-CreI, Sb/ TM6, Hu, Tb stock was used in Figure 3B (non-heat-shocked larvae were used as controls). w^{1118}; 70I-CreI, Sb/ TM6, Hu, Tb was used in Figures 1B–1D and 3A, Figure S2, and Table 1. y^1, w^{1118} larvae were used as controls in Figure 1 and Figure S2. y^1, w^{1118}; p[w^+; RFP-H2Av]; p[w^+; GFP-Polo], 70I-CreI, Sb/ TM6, Hu, Tb was used in Figure 3C (non-heat-shocked larvae were used as controls). To obtain *bubR1-KEN* mutant larvae with I-CreI used in Figures 4 and 5, Figure S2, and Table 1: females y^1, w^{1118}, p[w^+, RFP-BubR1-KEN]; $bubR1^1$ were crossed with males y^1, w^{1118}/ Y; $bubR1^1$/ CyO, y^+; 70I-CreI, Sb/ TM6, Hu, Tb. The yellow and non-Tb female larvae were selected for I-CreI expression. The heat-shocked yellow and Tb female larvae were used as controls. The *bubR1-KEN; bubR1*/+ described in Figure 5B are y^1, w^{1118}, p[w^+, RFP-BubR1-KEN]/ y^1, w^{1118} ; $bubR1^1$/CyO, y^+; 70I-CreI, Sb/ +. The larvae were selected for black hooks and absence of Tb. To obtain *bubR1-KD* mutant larvae described in Figures 4 and 5, females y^1, w^{1118}; $bubR1^1$; p[w^+, BubR1-KD]/TM6, Hu, Tb were crossed with males y^1, w^{1118} ; $bubR1^1$/ CyO, y^+; 70I-CreI, Sb/ TM6, Hu, Tb. The yellow non-Tb female larvae were selected for I-CreI expression. The heat-shocked yellow Tb female larvae were used as controls. To obtain $polo^1$/$polo^{10}$ mutant larvae described in Figure 5 and Table 1, females w^{1118}; $polo^1$/ TM6, Hu, Tb were crossed with males w^{1118} ; $polo^{10}$, 70I-CreI, Sb/ TM6, Hu, Tb or w^{1118} ; $polo^{10}$/ TM6, Hu, Tb. The female larvae w^{1118} ; $polo^{10}$, 70I-CreI, Sb/ $polo^1$ were selected for the absence of Tb. The heat-shocked female larvae w^{1118} ; $polo^{10}$/ $polo^1$ were used as controls. To obtain $mad2^p$ mutant larvae described in Table 1, females w^{1118}; $mad2^p$/ $mad2^p$ were crossed with males w^{1118} ; $mad2^p$, 70I-CreI, Sb/ TM6, Hu, Tb. The non-Tb female larvae w^{1118} ; $mad2^p$, 70I-CreI, Sb/ $mad2^p$ were selected. The non-heat-shocked w^{1118} ; $mad2^p$, 70I-CreI, Sb/ $mad2^p$ were used as controls in Table 1.

Cytology

For the survival rate analysis, crawling third instar larvae (at least nine per experiment) were heat shocked for one hour and let to develop into adulthood in a 25°C incubator on standard media. The adult flies that had died in the media at birth were not counted as adult survivors. To determine the frequency of anaphases with lagging chromatids, third instar larval brain were dissected in PBS and stained with aceto-orcein as described in (Karess and Glover, 1989). The preparations were observed in phase contrast using an Olympus BH-2 with a 100X objective. Images were taken with a Nikon Coolpix 995 camera. To analyze the X chromosome morphology in prometaphase cells after I-CreI induction, the brains were treated with 10^{-4}M colchicine diluted in PBS for 30 to 45 min and transferred to a 0.5% Sodium Citrate hypotonic solution for 5 to 7 min. The brains were fixed and squashed as described in (Cenci et al., 2003)

Live Analysis and Microscopy

Live analysis presented in Figures S1A–S1C and Figure S2B were performed using a wide-field fluorescence inverted Leica DMI6000B microscope equipped with a Hamamatsu ORCA C9100 EM-CCD camera and a 100X (N.A 1.4) lens and 1X binning. Z series images of 0.2μm intervals were acquired, processed and deconvolved with LAS AF6000 software. Additional image processing was done using Adobe Photoshop. Images presented in Figure S2A were acquired using a DIC Zeiss Axioskop II microscope equipped with a Zeiss Axiocam HRm camera and a 100X (N.A. 1.3) lens. The images were acquired using Zeiss Axiovision software and processed using Adobe Photoshop.

SUPPLEMENTAL REFERENCES

Cenci, G., Siriaco, G., Raffa, G.D., Kellum, R., and Gatti, M. (2003). The Drosophila HOAP protein is required for telomere capping. Nat. Cell Biol. *5*, 82–84.

Karess, R.E., and Glover, D.M. (1989). rough deal: a gene required for proper mitotic segregation in Drosophila. J. Cell Biol. *109*, 2951–2961.

Sullivan, W., Fogarty, P., and Theurkauf, W. (1993). Mutations affecting the cytoskeletal organization of syncytial Drosophila embryos. Development *118*, 1245–1254.TNQ

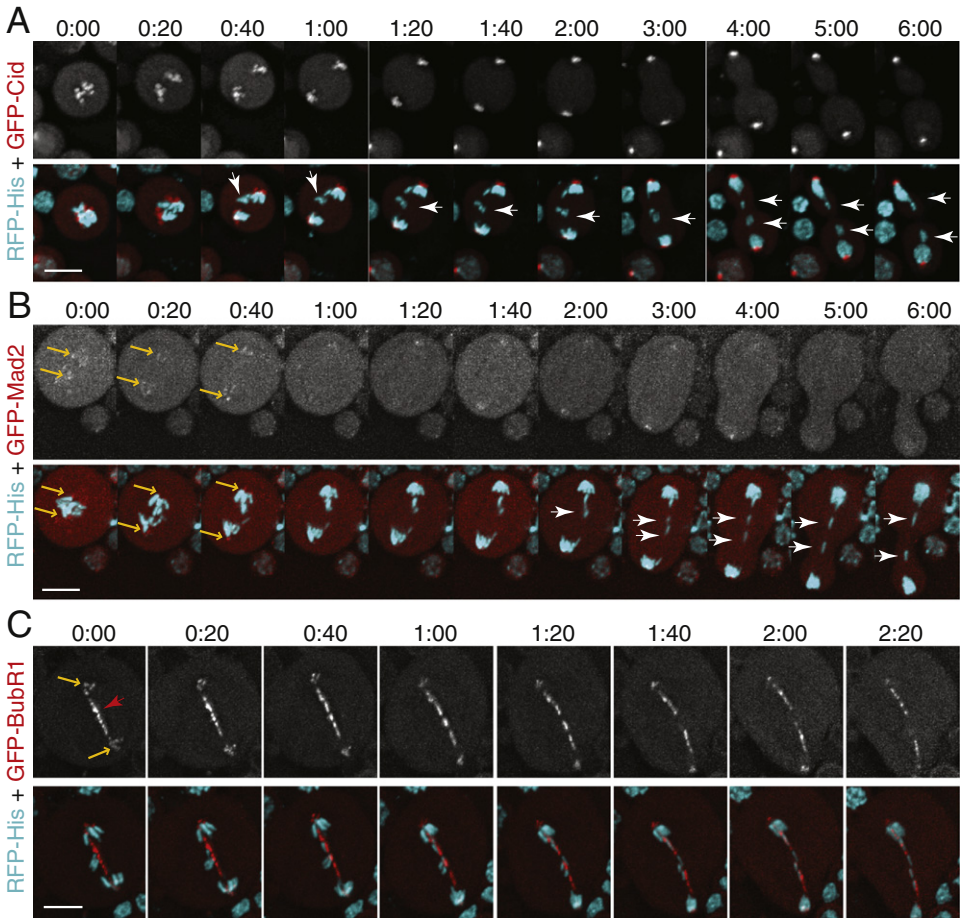

Figure S1. Unlike BubR1, Cid and Mad2 Do Not Localize on the Tether—Related to Figure 3

(A) Time-lapse imaging of neuroblasts from I-CreI heat-shocked larvae double labeled with GFP-Cid (red and top row) and RFP-Histone (cyan, bottom row). The white arrowheads indicate the position of the lagging acentrics. No GFP-Cid signal was detected on the lagging acentrics.

(B) Time-lapse imaging of neuroblasts from I-CreI heat-shocked larvae double labeled with GFP-Mad2 (red and top row) and RFP-Histone (cyan, bottom row). The yellow arrows highlight the localization of GFP-Mad2 at the kinetochore. The white arrowheads indicate the position of the lagging acentrics. No GFP-Mad2 labeling was detected on the lagging acentrics.

(C) Time-lapse imaging of neuroblasts from I-CreI heat-shocked larvae double labeled with GFP-BubR1 (red, top row) and RFP-Histone (cyan). The yellow arrows highlight BubR1 signal at the kinetochore. The red arrow points to BubR1 localization on the tether.

Each fluorescent image are deconvolved z projection.

Time: min:sec. Scale Bar: 10μm.

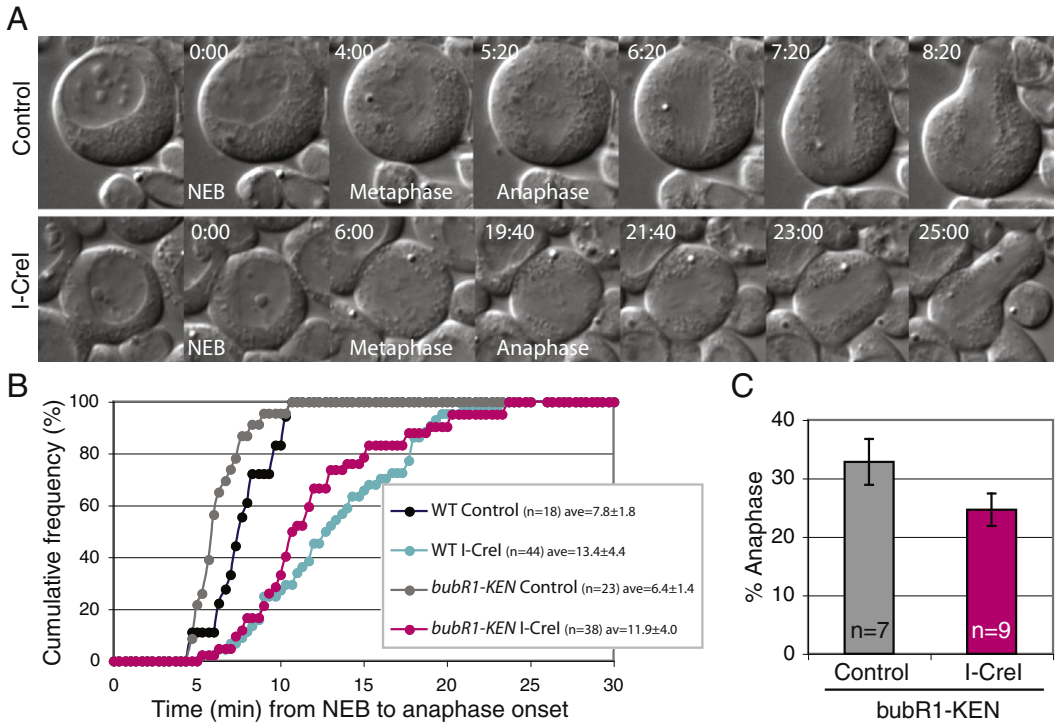

Figure S2. The Delay in Anaphase Onset after I-CreI Induction Does Not Depend on BubR1 Checkpoint Activity—Related to Figure 4

(A) Time-lapse imaging of neuroblasts from control and I-CreI heat-shocked larvae observed with DIC. Time: min:sec.

(B) Cumulative frequency of wild-type and *bubR1-KEN* cells that have entered anaphase at each time point (NEB = 0min). The graph shows the comparative mitotic transit from NEB to anaphase onset in control and I-CreI larvae for wild-type and *bubR1-KEN* mutant.

(C) Anaphase index in *bubR1-KEN* mutant with or without the *I-CreI* transgene (control and I-CreI respectively). The anaphase index was calculated as the number of anaphase/ number of mitotic neuroblasts. n = number of brains from 3 independent experiments for control and I-CreI. The total number of neuroblasts in mitosis scored for control and I-CreI were 1584 and 1888 respectively.