Kotadia et al., http://www.jcb.org/cgi/content/full/jcb.201208041/DC1



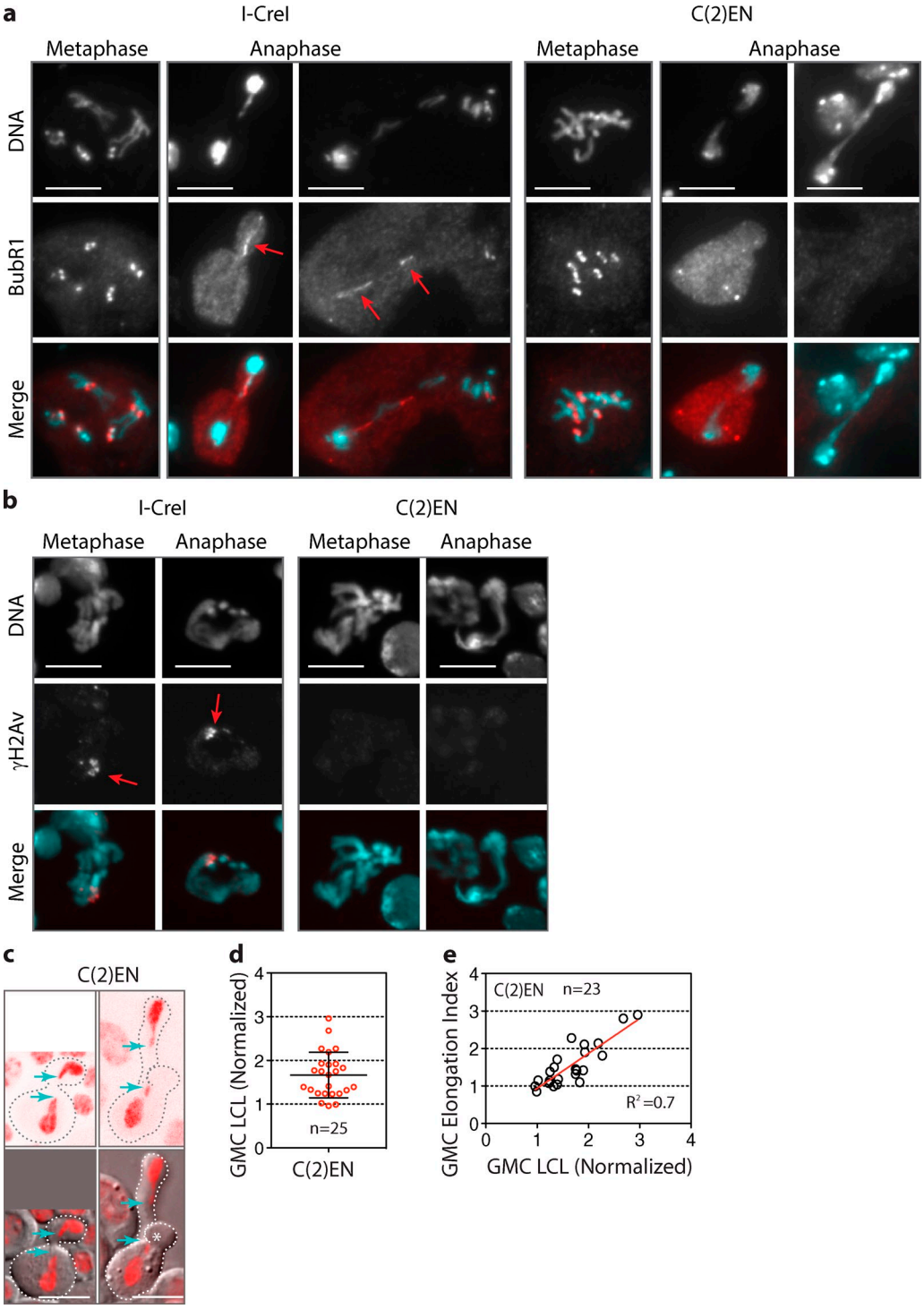



Figure S1. **The cell elongates during cytokinesis in the presence of a compound chromosome C(2)EN.** (a) DAPI (cyan) and anti-BubR1 (red) fluorescent images of fixed neuroblasts expressing I-CreI or carrying the C(2)EN compound chromosome. BubR1 is detected on kinetochores in metaphase cells expressing I-CreI or carrying C(2)EN. As previously described, BubR1 accumulates on the tether of I-CreI–induced acentric chromatids (red arrows). In contrast, no BubR1 signal is detected on segregating C(2)EN chromatid arms. Bars, 8 µm. (b) DAPI (cyan) and anti–γ-H2Av (red) fluorescent images of fixed neuroblasts expressing I-CreI or carrying C(2)EN. Consistent with previous observation, γ-H2Av that marks double-strand brakes is detected on the lagging chromatids in neuroblasts expressing I-CreI (red arrows; Royou et al., 2010). In contrast, no γ-H2Av signal is found on C(2)EN chromosome. Bars, 8 µm. (c) Still images from time-lapse movies of individual neuroblasts carrying a C(2)EN chromosome and expressing H2Av::RFP. Top panels are maximum projections of deconvolved H2Av::RFP images. Bottom panels are DIC images merged with the H2Av::RFP signal (red). The cyan arrows point to the tips of the lagging chromatids. The asterisk designates a cell bleb. Bars, 8 µm. (d) Scatter dot plot with mean ± SD showing the range of the longest chromatid length in the GMC (GMC LCL) for C(2)EN cells. (e) Graph showing the linear correlation ($R^2$ = 0.7) of the GMC elongation index with the length of the longest chromatid for C(2)EN cells. *n* = number of cells.

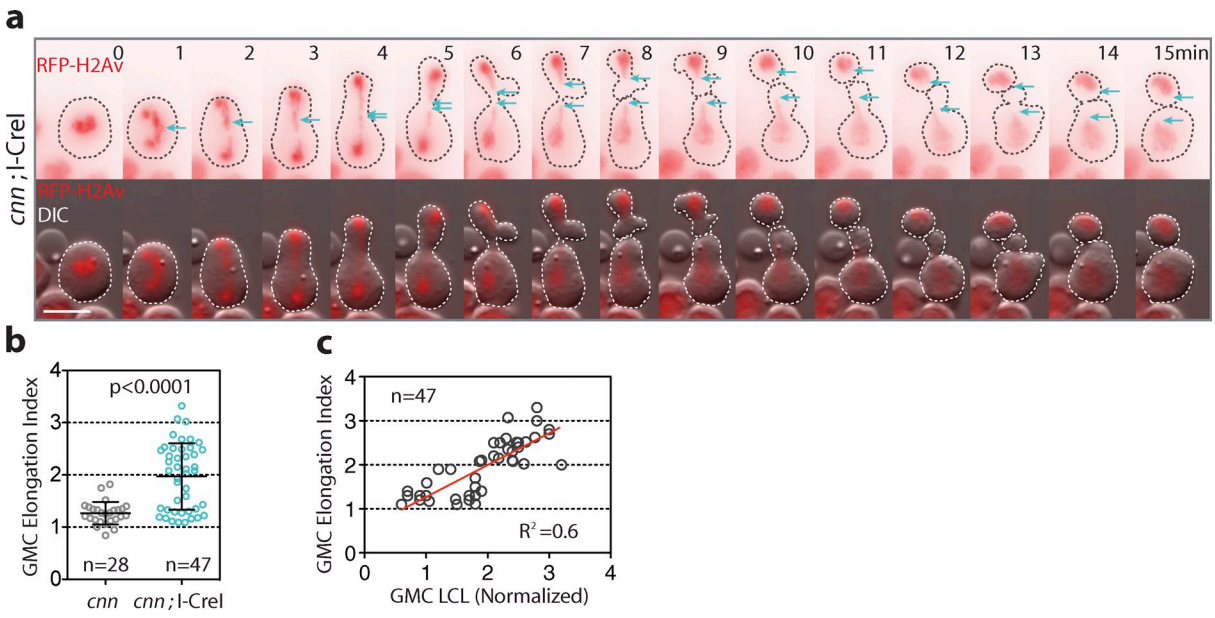



Figure S2. **Astral microtubules are not required for cell elongation during long chromatid segregation.** (a) Images of live *cnn* mutant neuroblasts expressing RFP::H2Av (red) from anaphase onset (time = 0 s) to the end of cytokinesis. Top panels are maximum projections of nondeconvolved H2Av::RFP images. Bottom panels are DIC images merged with H2Av::RFP signal (red). The broken lines outline the cell. The cyan arrows indicate the position of the tip of the longest chromatid. Bar, 8 µm. (b) Scatter dot plot with mean ± SD showing the GMC elongation index for control and I-CreI cells mutant for *cnn*. (c) Graph showing the linear correlation ($R^2$ = 0.6) of the GMC elongation index with the length of the longest chromatid for *cnn*; I-CreI cells. *n* = number of cells. An unpaired *t* test with 95% confidence was used to calculate the p-value.

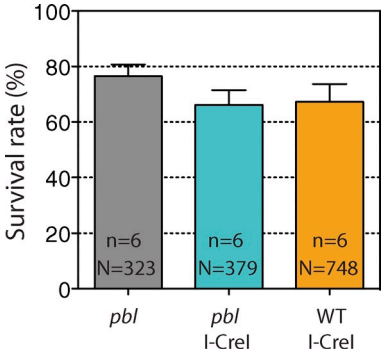
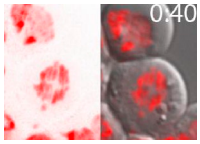
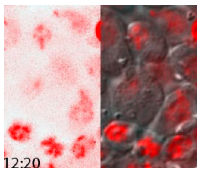


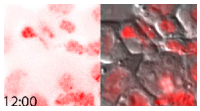

Figure S3. **No difference in the survival rate is observed between wild type and *pbl* mutant expressing I-CreI.** Histogram showing the frequency of survival to adulthood of heat-shocked third instar larvae. *n* = number of experiments. *N* = total number of heat shocked larvae.



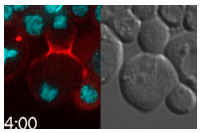

Video 1. **Wild-type neuroblast expressing H2Av::RFP.** Images were analyzed by time-lapse video-microscopy (Leica DMI6000B microscope equipped with a Hamamatsu ORCA 9100 EMCCD camera). Frames were taken every 20 s for 9 min. Left images are maximum projection of deconvolved H2Av::RFP signal (red). Right images are DIC merged with H2Av::RFP. The movie corresponds to Fig. 1 g, top row. Time is given in minutes:seconds.



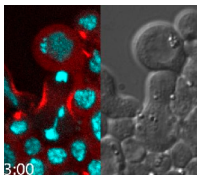

Video 2. **Wild-type neuroblast expressing H2Av::RFP and I-CreI.** Images were analyzed by time-lapse video microscopy (Leica DMI6000B microscope equipped with a Hamamatsu ORCA 9100 EMCCD camera). Frames were taken every 20 s for 12 min and 20 s. Left images are maximum projection of deconvolved H2Av::RFP signal (red). Right images are DIC merged with H2Av::RFP. The movie corresponds to Fig. 1 g, middle row. Time is given in minutes:seconds.



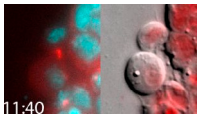

Video 3. **Wild-type neuroblast expressing H2Av::RFP and I-CreI.** Images were analyzed by time-lapse video microscopy (Leica DMI6000B microscope equipped with a Hamamatsu ORCA 9100 EMCCD camera). Frames were taken every 20 s for 12 min. Left images are maximum projection of deconvolved H2Av::RFP signal (red). Right images are DIC merged with H2Av::RFP. The movie corresponds to Fig. 1 g, bottom row. Time is given in minutes:seconds.




Video 4. **Wild-type neuroblast labeled with H2Av::RFP and RLC::GFP.** Images were analyzed by time-lapse video microscopy (Leica DMI6000B microscope equipped with a Hamamatsu ORCA 9100 EMCCD camera). Frames were taken every 20 s for 7 min and 20 s. Left images are maximum projection of deconvolved H2Av::RFP (cyan) and RLC::GFP (red) signals. Right images are DIC. The movie corresponds to Fig. 3 a, top row. Time is given in minutes:seconds.




Video 5. **Wild-type neuroblast labeled with H2Av::RFP and RLC::GFP (red) and expressing I-CreI.** Images were analyzed by time-lapse video microscopy (Leica DMI6000B microscope equipped with a Hamamatsu ORCA 9100 EMCCD camera). Frames were taken every 20 s for 16 min and 40 s. Left images are maximum projections of deconvolved H2Av::RFP (cyan) and RLC::GFP (red) signals. Right images are DIC. The movie corresponds to Fig. 3 a, bottom row. Time is given in minutes:seconds.




Video 6. ***pbl*$^{MS}$/*pbl*$^{5}$ mutant neuroblast labeled with H2Av::RFP and RCL::GFP and expressing I-CreI.** Images were analyzed by time-lapse video-microscopy (Zeiss Axio-observer microscope equipped with an evolve EMCCD camera; Photometrics). Frames were taken every 20 s for 16 min. Left images are maximum projection of nondeconvolved H2Av::RFP (cyan) and RLC::GFP (red) signals. Right images are DIC merged with H2Av::RFP signal (red). The movie corresponds to Fig. 4 a. Time is given in minutes:seconds.

## Reference

Royou, A., M.E. Gagou, R. Karess, and W. Sullivan. 2010. BubR1- and Polo-coated DNA tethers facilitate poleward segregation of acentric chromatids. *Cell*. 140:235–245. http://dx.doi.org/10.1016/j.cell.2009.12.043