Karg et al., https://doi.org/10.1083/jcb.201604079

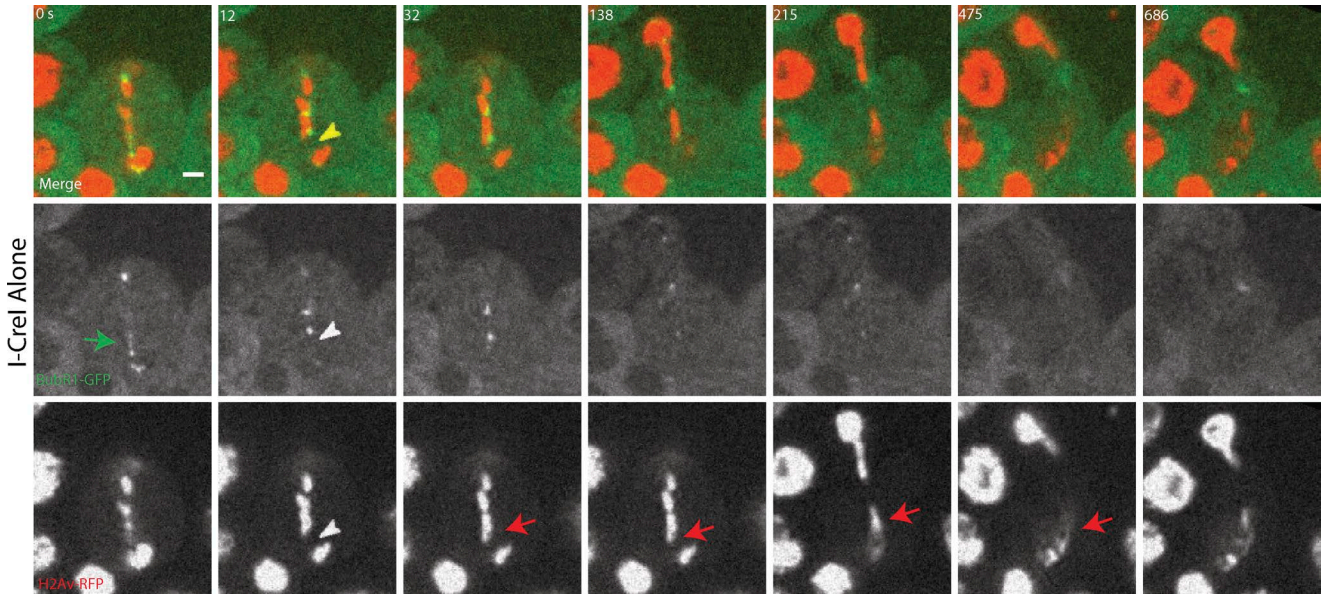


Figure S1. **Ablation of BubR1-coated tethers does not prevent poleward migration of acentrics.** Still images from a time-lapse movie (Video 6) of a mitotic neuroblast with I-CreI–induced acentrics connected to the main chromosome mass by a BubR1 coated tether (green arrow, 0 s). Arrowheads mark ablation spot of the tether (12 s). After ablation (32–686 s), acentric fragments (red arrows) migrate poleward and rejoin main chromosome mass. Bar, 2 µm.

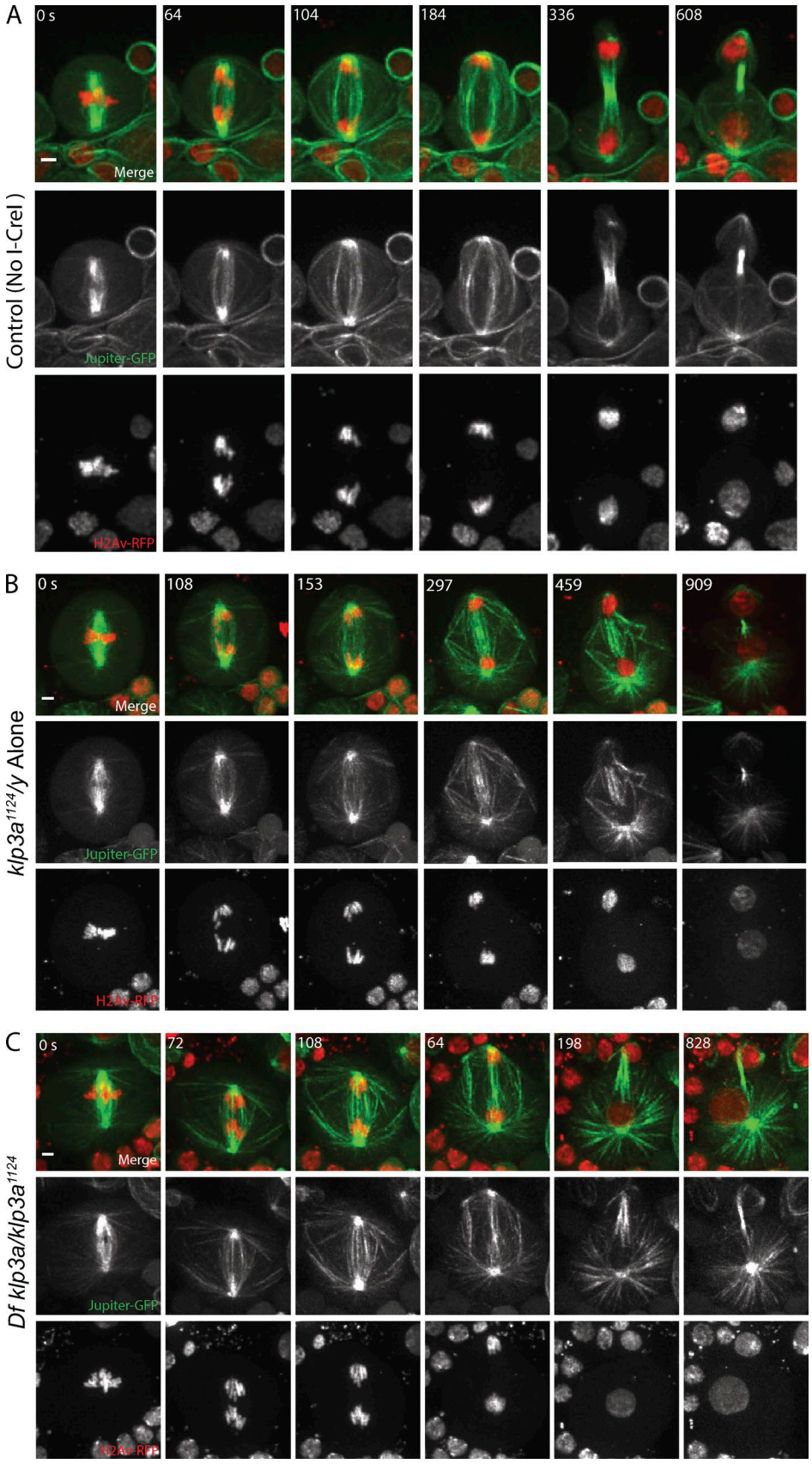


Figure S2. **Reduced Klp3a activity disrupts spindle morphology.** (A) Still images from a time-lapse movie of a control neuroblast showing chromosomes (red) and microtubules (green). (B and C) Still images from a time-lapse movie of a mitotic neuroblast division from a *Df klp3a/klp3a* and *klp3a*/y mutant third instars. Bars, 2 µm.

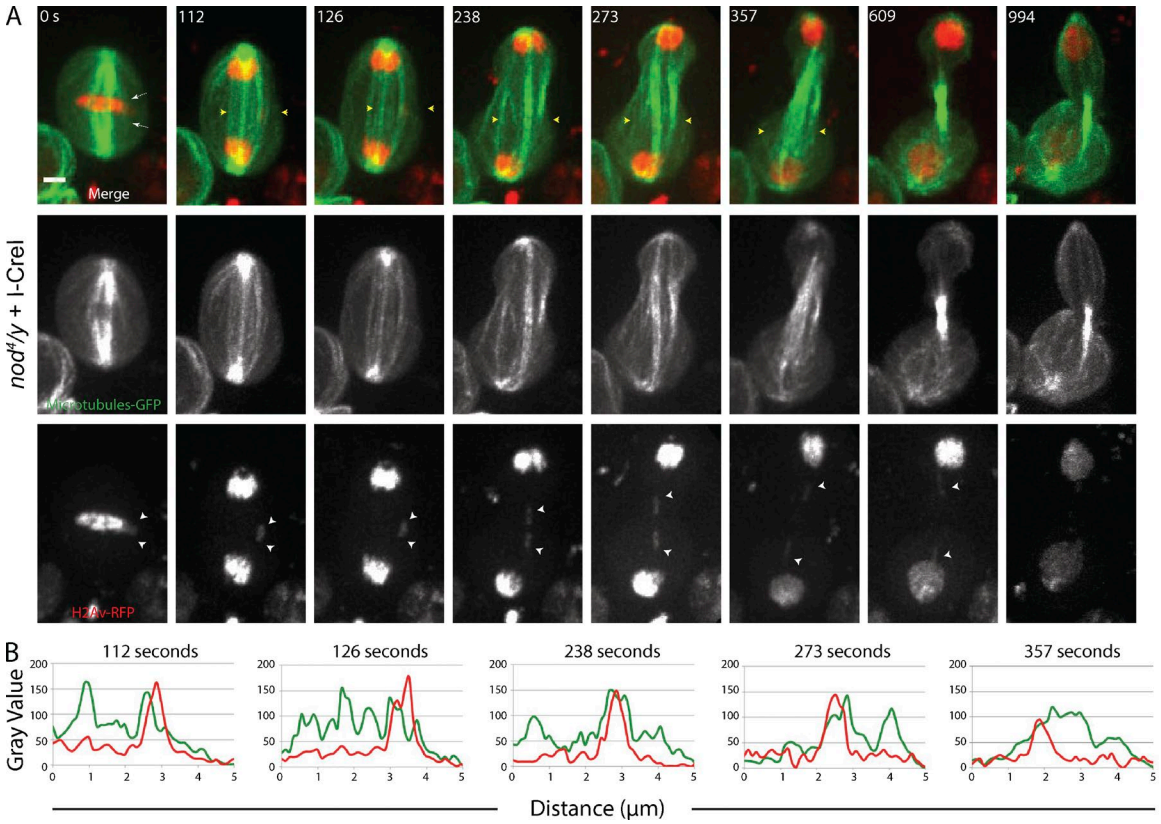


Figure S3. **Normal segregation of acentrics in neuroblasts from *nod*^{4} third instars.** (A) Still images from a time-lapse movie (Video 9) of a neuroblast from a *nod*^{4}/Y male with I-CreI–induced acentrics. Sister acentrics (red) are positioned to the edge of the metaphase plate (arrows) while in association with microtubules (green). By anaphase, sister acentrics (white arrowheads) separate and successfully migrate poleward while in association with microtubules. Bar, 2 μm. (B) Graphs showing the fluorescent intensities of microtubules (green line) and chromosomes (red line) in merged images at the time points 112, 126, 238, 273, and 357 s after anaphase. Line scans to measure fluorescent intensities were drawn between the yellow arrowheads in merged images.

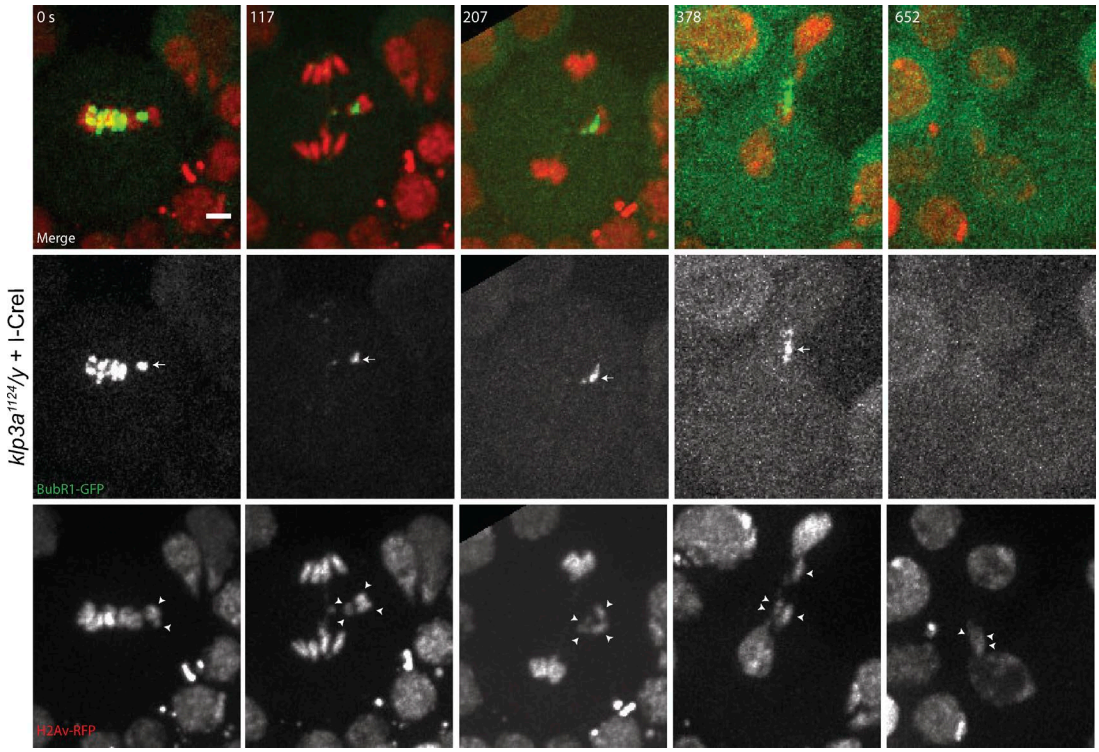


Figure S4. **Reduced Klp3a function does not disrupt BubR1 localization to DNA tether.** Still images from a time-lapse movie (Video 10) of a neuroblast from a *klp3a*^{1124}/Y male with I-CreI–induced acentrics (arrowheads) connected to the main chromosome mass by a BubR1-coated tether (arrow). Bar, 2 µm.

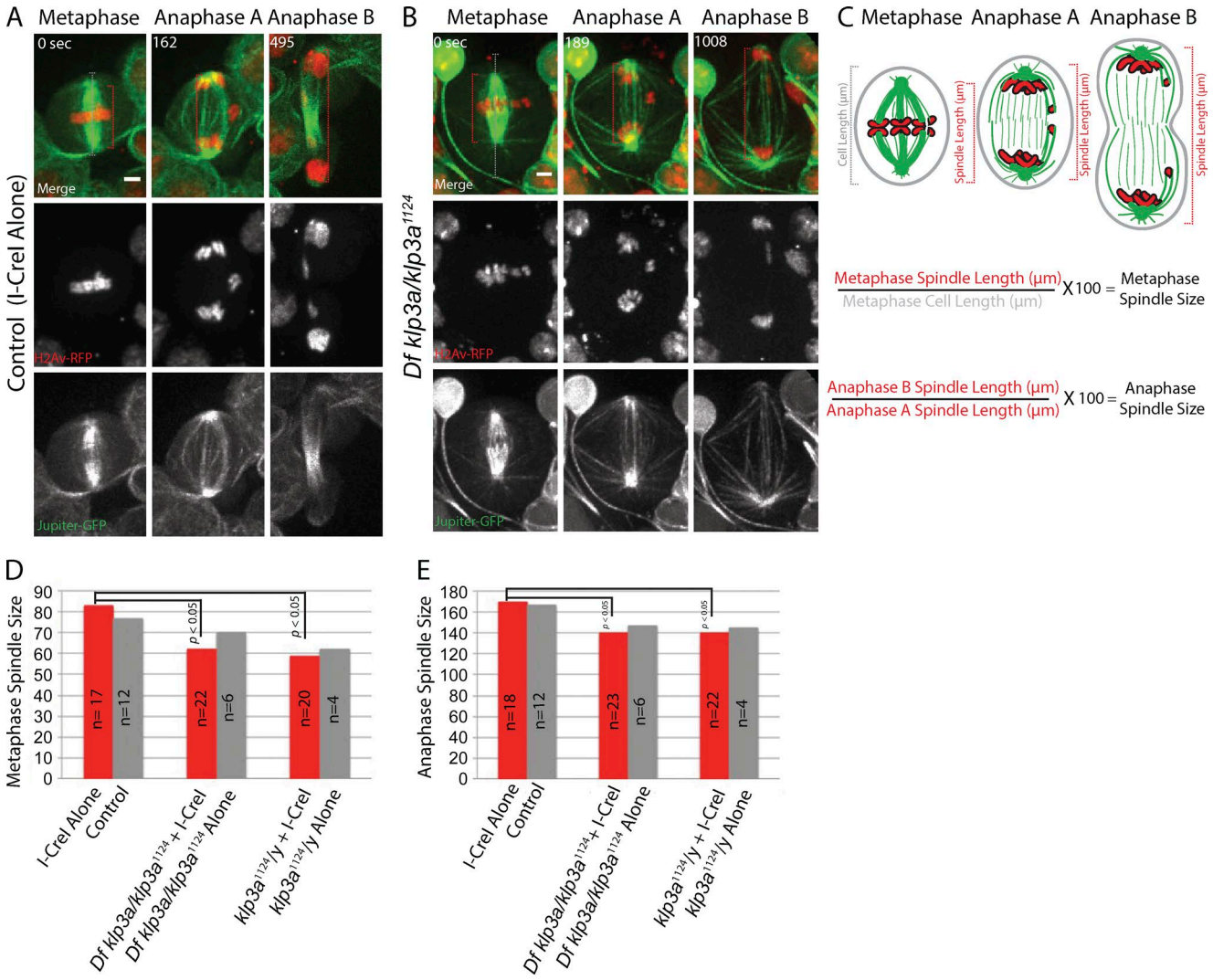
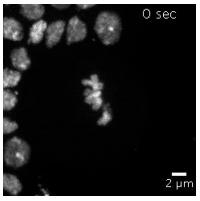
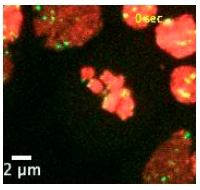


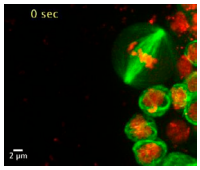
Figure S5. **Reduced metaphase spindle size and diminished anaphase elongation may contribute to acentric segregation defects in *klp3a* mutant neuroblast.** (A) Still images from a time-lapse movie of a control neuroblast showing chromosomes (red) and microtubules (green). (B) Still images from a time-lapse movie of a *Df klp3a/klp3a* mutant neuroblast showing reduced spindle size in metaphase and anaphase. Bars, 2 µm. (C) Schematic showing the calculation for measuring metaphase and anaphase spindle size. (D and E) Quantifications showing a statistically significant reduction in metaphase and anaphase spindle size in *Df klp3a/klp3a* and *klp3a*/Y mutant third instars.



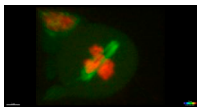
Video 1. **Third-instar neuroblast mitotic division with I-CreI–induced acentrics alone.** Chromosomes visualized by RFP-tagged histone variant H2Av. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 5 s for 40 min, 15 frames per second. Video corresponds to the still images from Fig. 1 B.



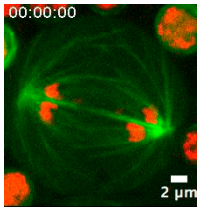
Video 2. **Third-instar neuroblast mitotic division with I-CreI–induced acentrics and GFP-tagged telomeres.** Chromosomes visualized by RFP-tagged histone variant H2Av, and telomeres visualized by GFP-tagged HOAP. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 5 s for 30 min, 15 frames per second. Video corresponds to the still images from Fig. 2 B.



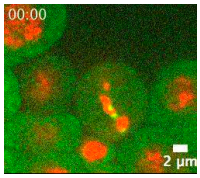
Video 3. **I-CreI–expressing third-instar neuroblast mitotic division with I-CreI–induced acentrics and GFP-tagged microtubules.** Chromosomes visualized by RFP-tagged histone variant H2Av, and microtubules are visualized by GFP-tagged microtubule associated protein called Jupiter. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 7 s for 23 min, 15 frames per second. Video corresponds to the still images from Fig. 3 B.



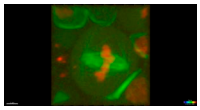
Video 4. **3D rendering of acentrics from an I-CreI–expressing third-instar neuroblast mitotic division with GFP-tagged microtubules.** Chromosomes visualized by RFP-tagged histone variant H2Av, and microtubules are visualized by GFP-tagged microtubule associated protein called Jupiter. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 7 s for 23 min, 15 frames per second. Video corresponds to the still images from Fig. 3 E.



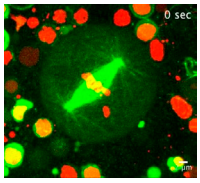
Video 5. **Third-instar neuroblast mitotic division with ablated (at time 00:00:45) GFP-tagged microtubules associated with I-CreI–induced acentrics.** Chromosomes visualized by RFP-tagged histone variant H2Av (red), and microtubules are visualized by GFP-tagged Jupiter (green). Images made by time-lapse spinning disc confocal microscope (Eclipse Ti-E). Frames taken every 3 s for 15 min, 15 frames per second. Time in minutes:seconds. Video corresponds to the still images from Fig. 4 A.



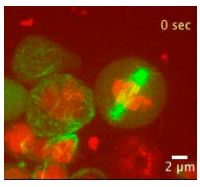
Video 6. **Third-instar neuroblast mitotic division with ablation (arrowhead at 00:33) of BubR1-coated tethers.** Chromosomes visualized by RFP-tagged histone variant H2Av (red), and BubR1 visualized by a GFP tag (green). Images made by time-lapse spinning disc confocal microscope (Eclipse Ti-E). Frames taken every 3 s for 15 min, 15 frames per seconds. Time in minutes: seconds. Video corresponds to the still images from Fig. S1.



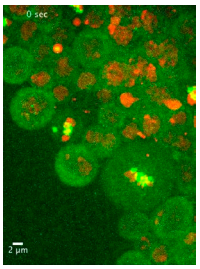
Video 7. **Third-instar neuroblast mitotic division from *klp3a*^{1124} with poleward acentrics.** Chromosomes visualized by RFP-tagged histone variant H2Av. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 5 s for 25 min, 15 frames per second. Video corresponds to the still images from Fig. 5 B.



Video 8. **3D rendering of a third-instar neuroblast mitotic division from *klp3a*^{1124} with poleward acentrics.** Chromosomes visualized by RFP-tagged histone variant H2Av. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 5 s for 25 min, 15 frames per second. Video corresponds to the still images from Fig. 6 C.



Video 9. **Third-instar neuroblast mitotic division from *nod*^{4} with I-CreI–induced acentrics.** Chromosomes visualized by RFP-tagged histone variant H2Av. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 7 s for 35 min, 15 frames per second. Video corresponds to the still images from Fig. S3.



Video 10. **BubR1-coated tethers form in I-CreI–expressing third-instar neuroblast from *klp3a*^{1124} mutants.** Chromosomes visualized by RFP-tagged histone variant H2Av and BubR1 visualized by GFP-tagged BubR1. Images made by time-lapse spinning disc confocal microscope (Eclipse TE2000-E). Frames taken every 5 s for 25 min, 15 frames per second. Video corresponds to the still images from Fig. S4.