Supplemental material

JCB



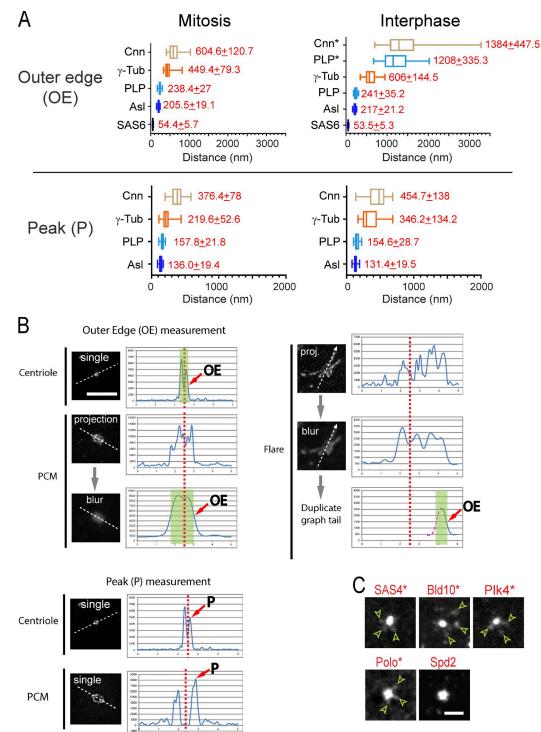
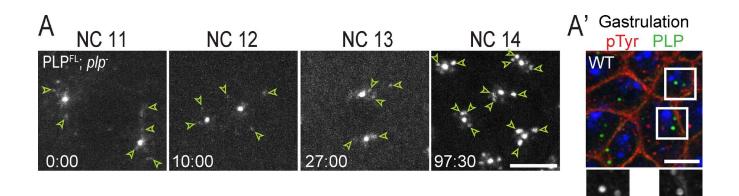


Figure S1. Analysis of centrosome protein distribution. (A) Quantification of WT mitotic (left) and interphase (right) centrosome protein distributions derived from SIM imaging of n = 30-110 centrosomes. Outer edge (OE) is the radial extension (relative to the centriole center) at half-maximal fluorescence intensity. The peak (P) is the radial intensity at the position of maximal fluorescence intensity relative to the centriole center (see Materials and methods). The asterisks denote flare-specific measurement. (B) Schematic showing processing of representative SIM images to define the OE and P. (C) The same confocal projections of the indicated proteins from Fig. 2 C are shown here with contrast enhancement to highlight the low fluorescence in the PCM and interphase flare zones (arrowheads). Endogenous Spd2 is not detected in the interphase flare zone, but is occasionally detected in the cytoplasm. Bars: (B) 2.5 μ m; (C) 1 μ m.



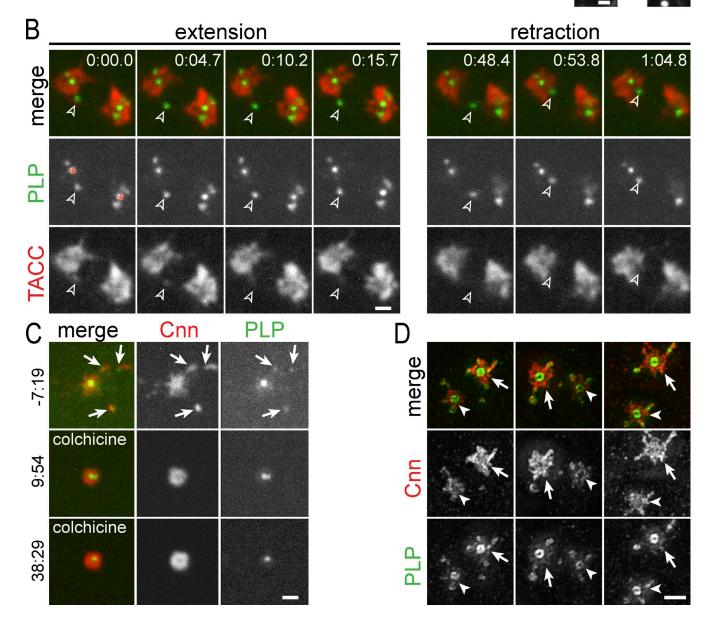


Figure S2. **Dynamics of PLP satellites.** (A). Live PLP^{FL} in successive NCs of the same embryo. Arrowheads show PLP satellites becoming more prominent with each cycle. Time is given in minutes:seconds. (A') Confocal projection showing interphase centrosomes (boxed sections enlarged below) in a gastrulating WT embryo stained for phospho-Tyrosine (pTyr) and PLP. (B) Live PLP^{FL} and TACC-RFP. Arrowheads show PLP satellite during an extension and retraction event. TACC is only found within the satellite briefly during extension. Time is given in minutes:seconds. (C) Live PLP^{FL} and Cnn-mCherry before and after colchicine injection (at t = 0 min). Arrows show flares and particles. (D) SIM image gallery of three mother (arrows) and daughter (arrowheads) centrosome pairs. Note that the dimmer Cnn signal in the daughter centrosome correlates with the dimmer PLP signal. Bars: (A and A', top) 5 µm; (A', bottom, and B–D) 1 µm.

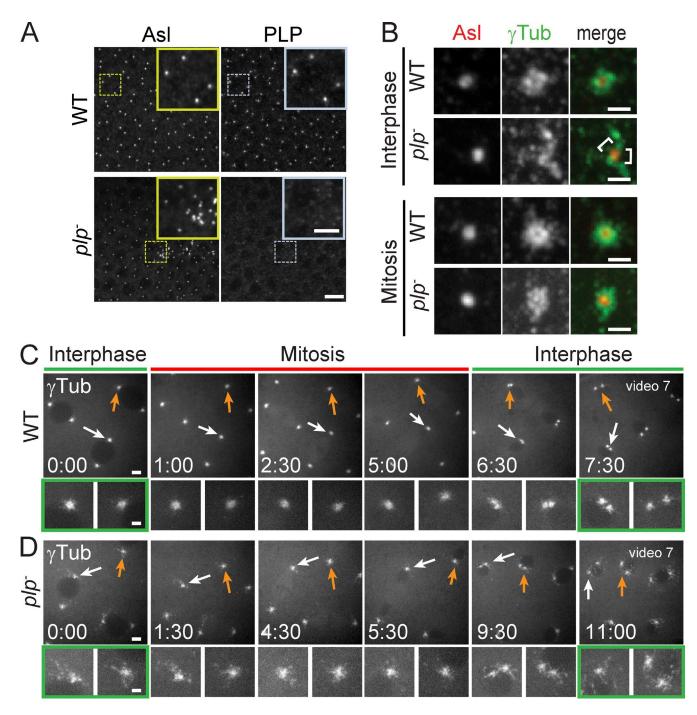


Figure S3. **Disruption of** γ **Tub in PLP germline clone embryos.** (A) PLP germline clone embryos ($p!p^-$) indicating the presence of centrioles (Asl staining) and the absence of PLP protein. Insets indicate normal centriole distribution in WT and clustered centrioles in $p!p^-$, a hallmark of NUF. The boxed regions are enlarged in the insets. (B) WT and $p!p^-$ embryos stained for γ -Tub show that PCM disruption is found specifically in interphase centrosomes. Brackets highlight discontinuities/breaks in the PCM. (C and D) Live GFP- γ Tub in WT and $p!p^-$ embryos. Bottom panels show enlarged views (400%) of the centrosomes indicated by the arrows (white, left; orange, right). Time is given in minutes:seconds. Comparison of the green boxes highlights the disruption of γ -Tub in interphase $p!p^-$ embryos. Bars: (A, main panels) 2.5 µm; (A, insets) 1 µm; (B) 1 µm; (C and D, top) 5 µm; (C and D, bottom) 1 µm.

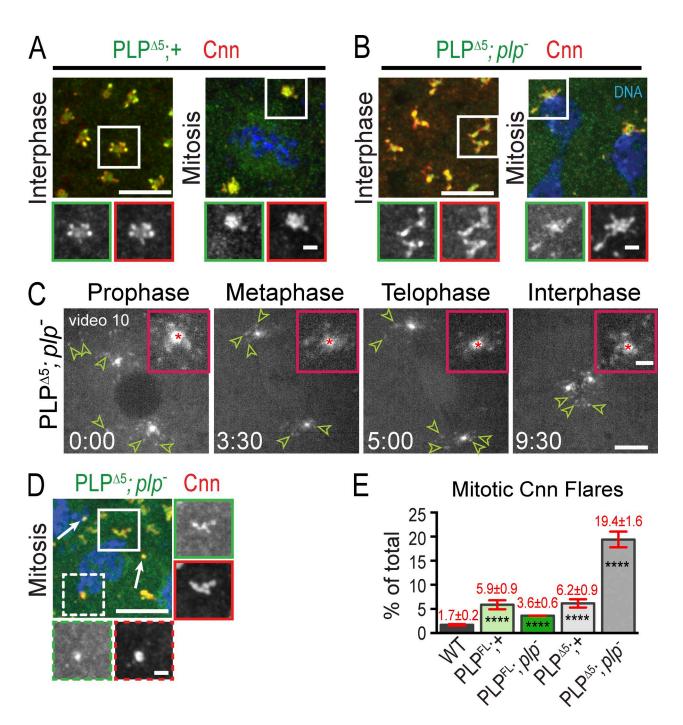
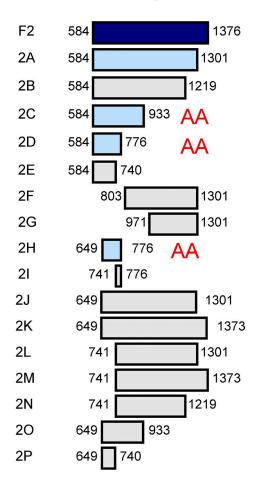
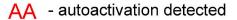


Figure S4. **PLP-F5 is required for compaction of the mitotic centrosome.** (A) $PLP^{\Delta 5}$ -GFP expressed in WT embryos show no dominant effects on Cnn (red) in interphase or mitosis. The boxed regions are enlarged below. (B) $PLP^{\Delta 5}$ -GFP expressed in p/p^{-} embryos results in abnormal interphase flares that persist in mitosis. (C) Live $PLP^{\Delta 5}$ -GFP expressed in p/p^{-} localizes to centrioles (asterisk) and satellites (arrowheads) throughout the cell cycle. Time is given in minutes:seconds. (D) $PLP^{\Delta 5}$ embryos stained for Cnn show the intimate relationship between the PLP satellites and Cnn flares. When satellites are absent, so are flares (broken box and bottom panels). When satellites are present, so are flares (solid box and right panels). Arrows show cytoplasmic particles. (E) Frequency of interphase-like Cnn flares in mitotic embryos. n > 350 centrosomes and ≥ 5 embryos for each genotype. Data are mean \pm SD (error bars) from two independent experiments. ****, P < 0.0001 for all comparisons by ANOVA. Bars: (A–D, main panels) 5 µm; (A–D, enlarged panels); 1 µm.

PLP-F2 Subfragments used in Y2H





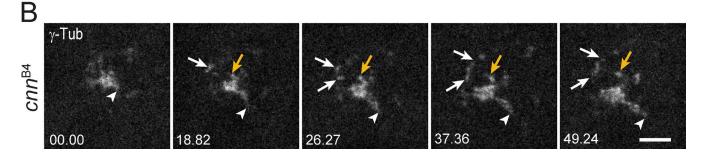
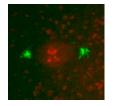


Figure S5. Interaction refinement of PLP-F2. (A) Graphic showing truncations of PLP-F2 (dark blue) used in Y2H analysis. Color indicates the fragments that did (light blue) or did not (gray) interact with Cnn-F3. Fragments that displayed autoactivation (AA) are indicated. We identified PLP-F2A (aa 584–1301) as the sole truncation of PLP-F2 capable of interacting with Cnn-F3 without autoactivation. (B) Live GFP-γTub in a *cnn*^{B4} embryo. Arrows show γTub diffusing away from the centrosome, arrowheads show a fiber of γTub extending into the cytosol. Time is given in seconds. Bar, 2.5 µm.

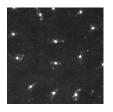
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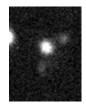
Video 1. Cnn-GFP and H2A-RFP through multiple NCs in a control embryo. Live control *Drosophila* embryo expressing Cnn-GFP (green) and H2A-RFP (red). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were captured every 10 s for 26.5 min.



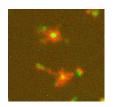
Video 2. **Cnn-GFP during the mitosis-to-interphase transition in a control embryo.** Live control *Drosophila* embryo expressing Cnn-GFP (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 300 ms for 5 min.



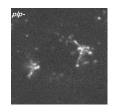
Video 3. **PLP**^{FL} in a cycling *plp*⁻ embryo. Live *plp*⁻ *Drosophila* embryo expressing PLP^{FL}-GFP (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 30 s for 20.5 min.



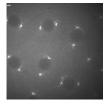
Video 4. **PLP satellite dynamics.** Live *plp⁻ Drosophila* embryo expressing PLP^{FL}-GFP (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 500 ms for 30 s.



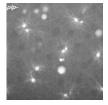
Video 5. **PLP^{FL} and Cnn-mCherry are packaged as dynamic particles.** Live control *Drosophila* embryo expressing PLP^{FL}GFP (white) and Cnn-mCherry (red). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 1 s for 30 s.



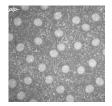
Video 6. **Cnn-mCherry in a control and** plp^- embryo. Live control and plp^- *Drosophila* embryos expressing Cnn-mCherry (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 500 ms for 30 s.



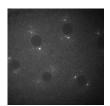
Video 7. **GFP-yTub through multiple NCs in a control and** p/p^- **embryo.** Live control and p/p^- *Drosophila* embryos expressing GFP-yTub (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 30 s for 21 min for WT and 27 min for the mutant.



Video 8. **GFP-MT in a control and** *plp*⁻ **embryo.** Live control and *plp*⁻ *Drosophila* embryos expressing GFP-MT (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 30 s for 15 min.



Video 9. **H2A-RFP in a control and** *plp*⁻ **embryo.** Live control and *plp*⁻ *Drosophila* embryos expressing H2A-RFP (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 30 s for 15 min for WT and 17.5 min for the mutant.



Video 10. **PLP^{Δ5} in a cycling** *plp*⁻ **embryo.** Live *plp*⁻ *Drosophila* embryo expressing PLP^{Δ5}-GFP (white). Images were analyzed by time-lapse spinning disk confocal microscopy (Ti Eclipse; Nikon). Frames were taken every 30 s for 18.5 min.