

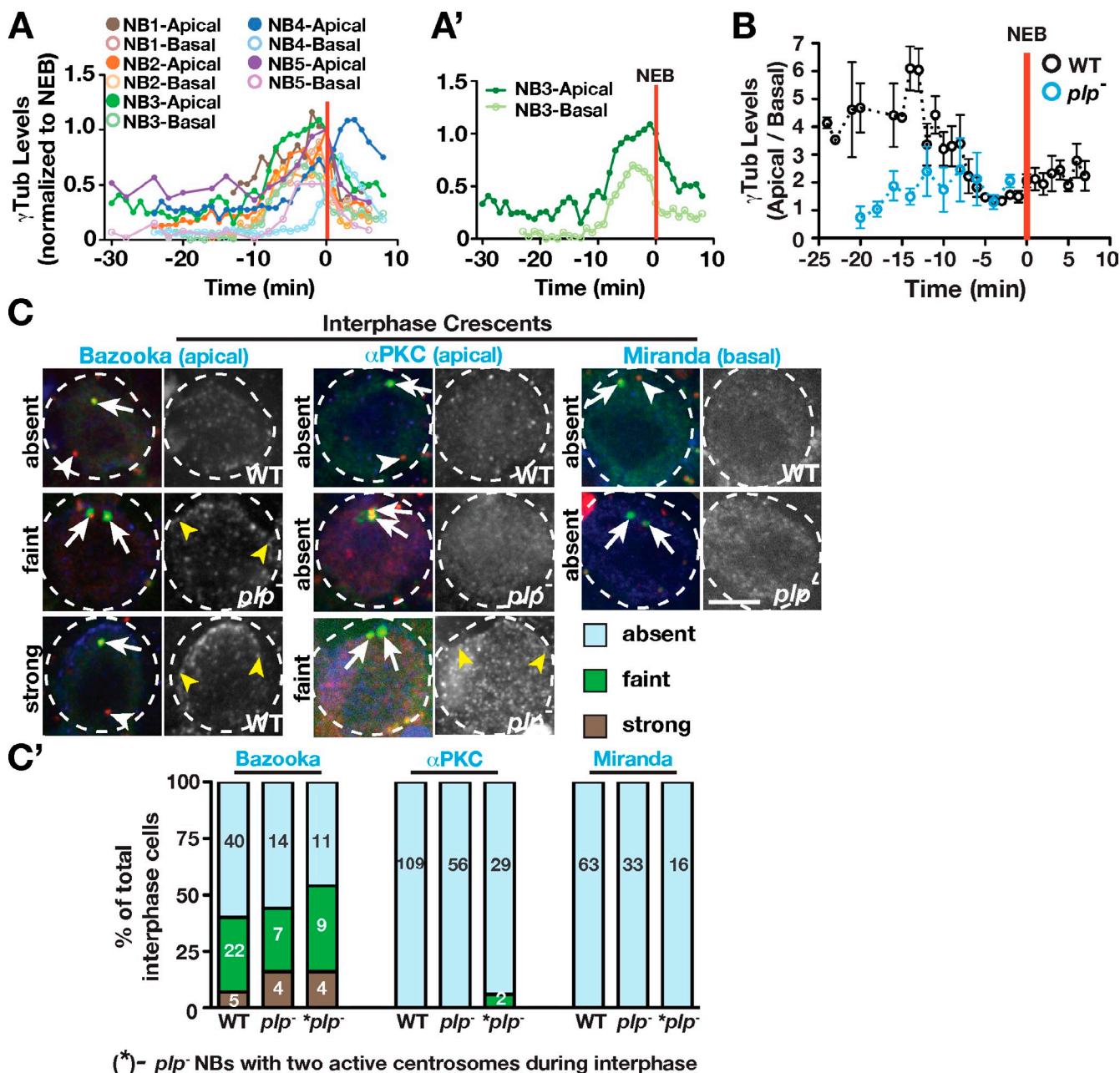
Lerit and Rusan, <http://www.jcb.org/cgi/content/full/jcb.201303141/DC1>

Figure S1. **PLP restricts maturation of the interphase mother centrosome.** (A and A') Quantification of γ -Tub levels on the apical and basal centrosomes in cycling control NBs with times normalized to NEB. Panel A shows superimposed data from five WT NBs, and A' is an isolated example. (B) Plot of the (A/B) enrichment of γ -Tub on WT and *plp*⁻ centrosomes. Each data point (\pm SEM) represents the mean of several measurements for the indicated time points. (C and C') Interphase localization of apical (Baz; α -PKC) and basal (Mira) polarity (yellow arrowheads define the edges of the polarity crescent) was characterized as strong, faint, or absent and quantified in C'. **plp*⁻ NBs contain two apical γ -Tub-positive centrosomes. The number of NBs analyzed is indicated. In all panels, apical (arrows) and basal (white arrowheads) centrosomes are indicated. Dashed circles, NBs. Bar, 5 μ m.

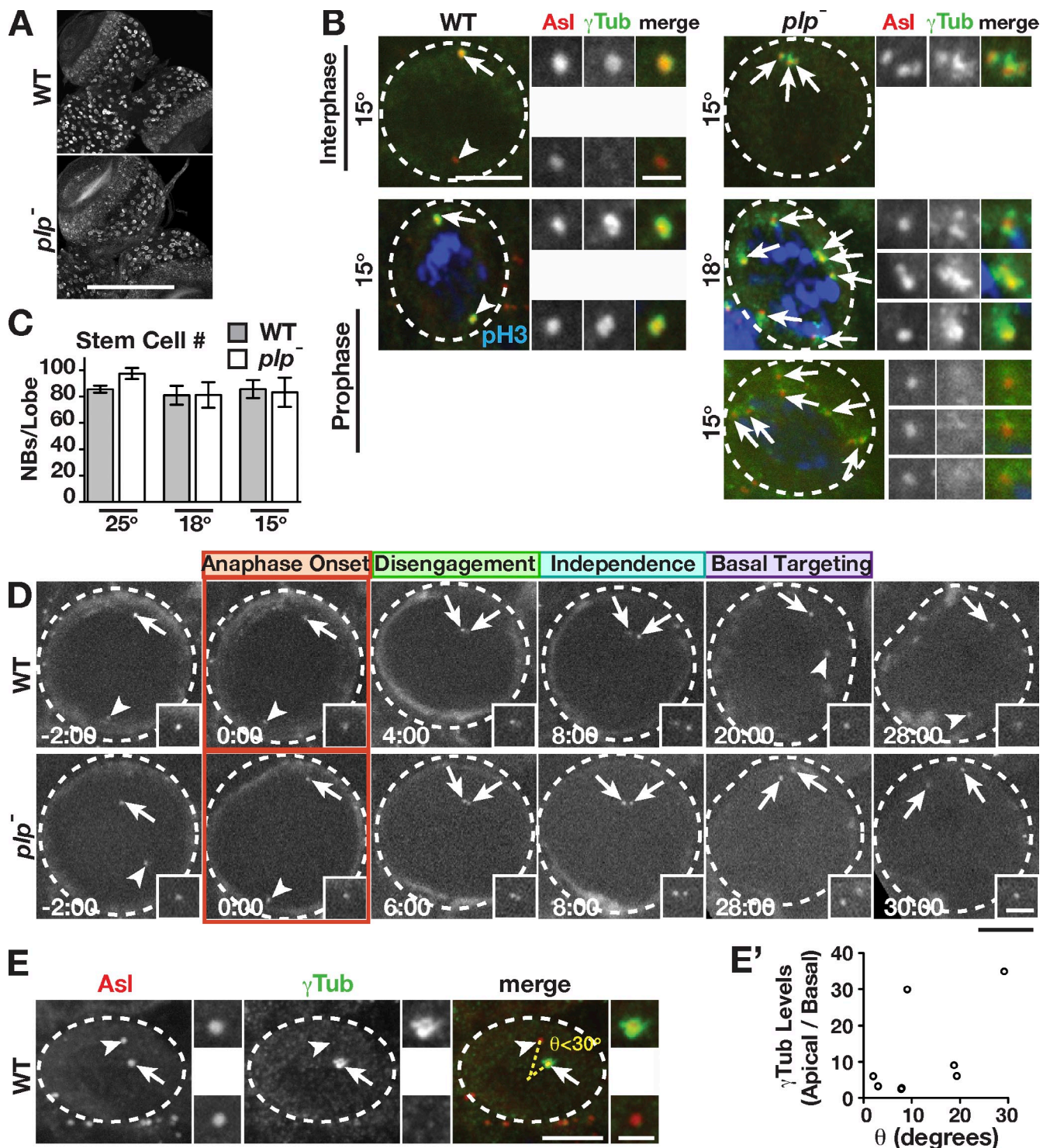
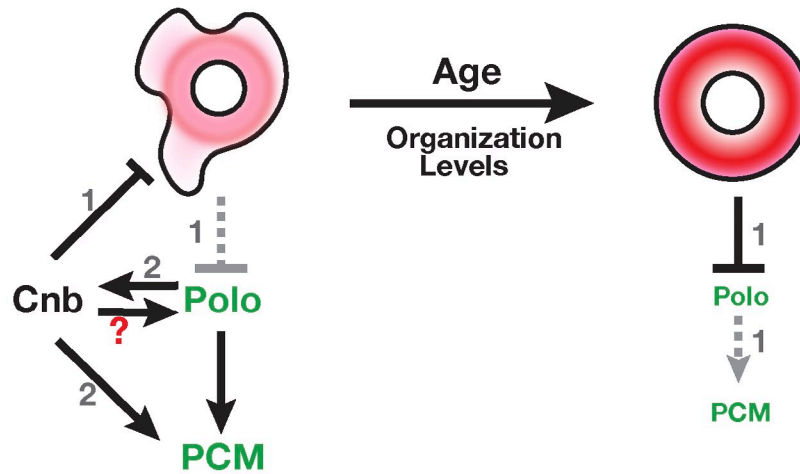


Figure S2. Cold sensitization of NBs. (A) Whole brains were stained for Mira to count NBs. (B) Projections of supernumerary centrosomes in cold-treated NBs. In WT NBs, apical (white arrows) and basal (white arrowheads) centrosomes are indicated, whereas in *plp*⁻ NBs, all centrosomes are highlighted with white arrows. (C) Quantification of stem cell number in brains from the indicated genotypes reared at the given temperatures is shown as means \pm SD. Number of brains analyzed were as follows: 25°C (WT: $n = 5$; *plp*⁻: $n = 4$), 18°C (WT: $n = 9$; *plp*⁻: $n = 9$), and 15°C (WT: $n = 10$; *plp*⁻: $n = 8$). (D) Live GFP-SAS6 and GFP-Moesin (Moe) in NBs. Apical (arrows; inset) and basal (arrowheads) centrosomes are shown. Times are minutes and seconds relative to anaphase (red boxes). Approximate indication of disengagement, the time of the first clear separation of the two centrosomes; independence, the time of the first indication of independent centrosome movement (remain apical); and basal targeting, the first indication of the active movement of one of the centrosomes toward the basal side of the NB are indicated above each corresponding image. Number of NBs analyzed from two to three brains were as follows: disengagement (WT: $n = 13$; *plp*⁻: $n = 9$) and independence (WT: $n = 11$; *plp*⁻: $n = 9$). Disengagement and independence were not significantly different. Basal targeting (25.3 ± 3.6 for control vs. 30.5 ± 6.2 for mutant) was longer in *plp*⁻ NBs ($P < 0.01$, two-tailed Student's t test). The mean for basal targeting in mutant cells includes NBs with one and two active centrosomes. (E) Projection of a WT NB showing asymmetric γ -Tub localization and $\theta < 30^\circ$. (E') Quantification of the relative enrichment of γ -Tub on centrosomes from NBs ($n = 8$, 4 brains from one experiment) with $\theta < 30^\circ$. The adjacent boxes (grayscale) are magnifications of the indicated proteins showing the apical (top) and basal (bottom) centrosomes. Dashed circles, NBs. Bars: (A) 100 μ m; (B, D, and E, main images) 5 μ m; (B, D, and E, insets) 1.5 μ m.

A**Interphase Centrosome Maturation**

Apical/Daughter Centrosome (Active) **Basal/Mother Centrosome (Inactive)**



1 This Study
2 Januschke et al., 2013

PLP levels**B**

Interphase NEB Metaphase Interphase

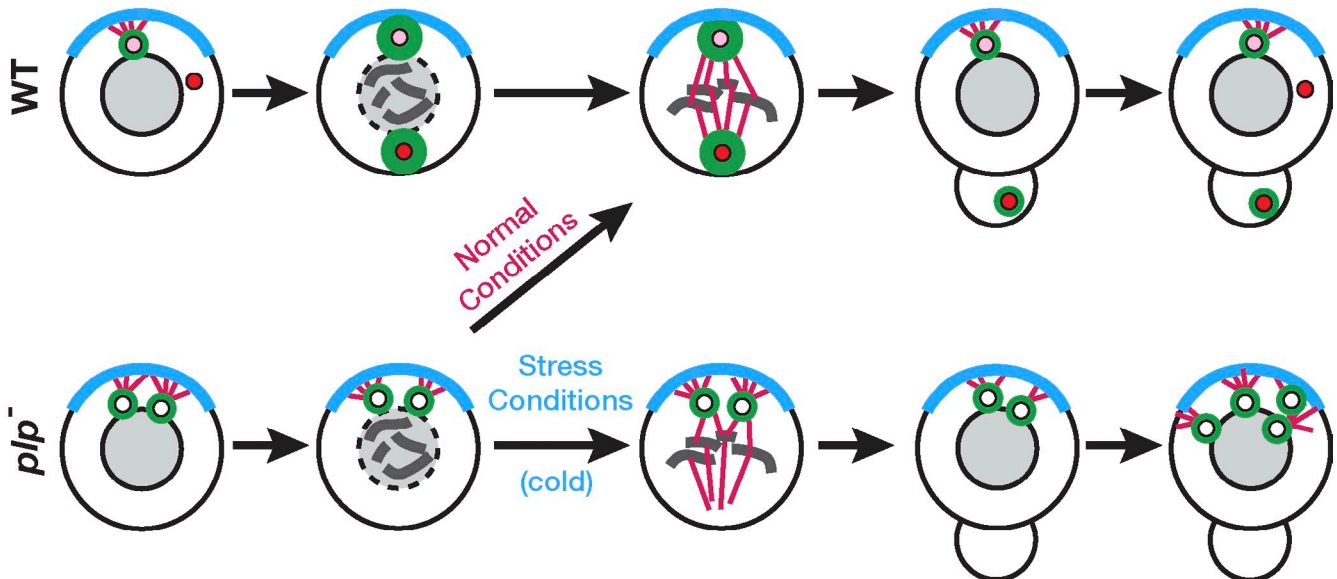
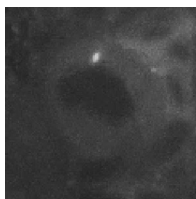
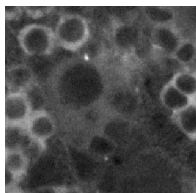


Figure S3. **Proposed model.** (A) During normal development, interphase NB centrosomes are asymmetric. The apical/daughter centrosome is active and recruits PCM in a Polo-dependent manner (green). The basal/mother centrosome, however, is inactive. We show that PLP (red) is localized to both centrosomes, but it is enriched on the inactive one and prohibits the localization of Polo and PCM. PLP apical must be distinct to allow for centrosome maturation. PLP basal may block centrosome maturation because of either (a) increased levels of PLP, (b) an organization of PLP that blocks PCM recruitment, (c) differential turnover of PLP itself or of its binding partners, and/or (d) by posttranslational modification of PLP. Upon mitotic onset, both PLP pools may attain apical traits. Cnb is sufficient to displace PLP and activate the centrosome. See text related to Fig. 3 for details. (B) In contrast to WT NBs, *plp*⁻ NBs contain two active centrosomes at the apical domain, presumably because their MTs (red) allow both centrosomes to attach to the apical cortex (blue). Usually, the position of the *plp*⁻ centrosomes is corrected after NEB, and each cell receives the normal centrosome number. Under stress, *plp*⁻ NBs receive the improper centrosome number.



Video 1. **GFP- γ -Tub23C in a cycling control NB.** Related to Fig. 2 A. Dividing NBs expressing GFP- γ -Tub (white) were imaged by time-lapse spinning-disk confocal microscopy. Frames are shown as projections and were captured at 2-min intervals for 42 min.



Video 2. **GFP- γ -Tub23C in a cycling *plp*⁻ NB.** Related to Fig. 2 B. Dividing NBs expressing GFP- γ -Tub (white) were imaged by time-lapse spinning-disk confocal microscopy. Frames are shown as projections and were captured at 2-min intervals for 50 min.

Reference

Januschke, J., J. Reina, S. Llamazares, T. Bertran, F. Rossi, J. Roig, and C. Gonzalez. 2013. Centrobin controls mother-daughter centriole asymmetry in *Drosophila* neuroblasts. *Nat. Cell Biol.* 15:241–248. <http://dx.doi.org/10.1038/ncb2671>