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Report

The Centriolar Protein Bld10/Cep135 Is Required to Establish Centrosome Asymmetry in *Drosophila* Neuroblasts

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Summary

Centrosome asymmetry has been implicated in stem cell fate maintenance in both flies and vertebrates [1, 2]. *Drosophila* neuroblasts, the neural precursors of the fly's central nervous system [3], contain molecularly and physically asymmetric centrosomes, established through differences in pericentriolar matrix (PCM) retention [4–7]. For instance, the daughter centriole maintains PCM and thus microtubule-organizing center (MTOC) activity through Polo-mediated phosphorylation of Centrobin (Cnb) [7, 8]. The mother centriole, however, quickly downregulates PCM and moves away from the apical cortex, randomly migrating through the cytoplasm until maturation sets in at prophase [4–6, 8]. How PCM downregulation is molecularly controlled is currently unknown, but it involves Pericentrin (PCNT)-like protein (PLP) to prevent premature Polo localization and thus MTOC activity [9]. Here, we report that the centriolar protein Bld10, the fly ortholog of Cep135, is required to establish centrosome asymmetry in *Drosophila* neuroblasts through shedding of Polo from the mother centrosome. *bld10* mutants fail to downregulate Polo and PCM, generating two active, improperly positioned MTOCs. Failure to shed Polo and PCM causes spindle alignment and centrosome segregation defects, resulting in neuroblasts incorrectly retaining the older mother centrosome. Since Cep135 is implicated in primary microcephaly, we speculate that perturbed centrosome asymmetry could contribute to this rare neurodevelopmental disease.

Results and Discussion

In a gene candidate approach to identify molecules required for centrosome asymmetry in *Drosophila* neuroblasts, we identified Bld10/Cep135 as a potential centrosome dematuration regulator. Bld10 is a ubiquitous centriolar protein, localizing to centrioles in *Drosophila* larval neuroblasts and other cell types ([10] and data not shown).

To investigate centrosome asymmetry, we performed live imaging experiments in intact third-instar larval brains (see the Supplemental Experimental Procedures and [11]), labeling centrosomes with the centriolar markers DSas6::GFP [12] or DSas4::GFP [13] and mCherry::Jupiter [14]. *jupiter* encodes for a microtubule binding protein, sharing properties with several structural microtubule-associated proteins (MAPs) [15], and is ideally suited to visualize microtubule dynamics and microtubule-organizing center (MTOC) activity. In agreement with previous findings [4–6], we found that wild-type (WT) interphase neuroblasts contained one apical MTOC only. The second MTOC appeared during prophase in close proximity to the basal cortex. By prometaphase, both MTOCs reached maximal activity and intensity (Figures 1A and 1B, time point 0:00). However, in *bld10* mutant interphase neuroblasts ($bld10^{c04199}$/*Df(3L)Brd15*; see the Supplemental Experimental Procedures and [10]), we observed two centrosomes of similar size and MTOC activity close together on the apical cortex. The two centrosomes progressively separated from each other until they reached their respective positions on the apical and basal cortex by prometaphase (Figures 1C and 1D, time point 0:00). Thus, in contrast to the wild-type, *bld10* mutant neuroblasts show symmetric centrosome behavior. *bld10*'s centrosome asymmetry defect could be rescued with *bld10::GFP* [12] (Figure 1E), and immunohistochemistry experiments confirmed our live imaging results (data not shown).

The $bld10^{c04199}$ allele is predicted to produce a truncated protein, retaining Bld10's N terminus [10]. We generated a new N-terminal deletion allele (*bld10ΔN*; see the Supplemental Experimental Procedures and Figures S1A and S1B) [16] that showed the same centrosome asymmetry phenotype (Figures S1C and S1D). In addition, we also found neuroblasts containing monopolar and multipolar spindles (Figures S1D–S1H), which are not observed with the $bld10^{c04199}$ allele. This suggests that $bld10^{c04199}$ is a separation-of-function allele, specifically disrupting centrosome asymmetry. Unless otherwise noted, all of the experiments described in the following sections were performed with the $bld10^{c04199}$ allele.

The lack of centrosome asymmetry in *bld10* mutant neuroblasts could be due to aberrant centriole migration. For example, the mother centriole could either fail to migrate through the cytoplasm or migrates back to the apical cortex to mature. We tested this hypothesis, measuring centriole migration as a function of time and observed that centriolar migration in wild-type and *bld10* mutant neuroblasts occur in two distinct phases: (1) centrioles steadily separated from each other, followed by (2) a sudden increase in intercentriolar distance, which peaked when centrioles reached a separation distance of ~4–6 μm in the wild-type and *bld10* mutants (Figure 1F). Centrioles in *bld10* mutants did not require more time to reach this threshold distance (Figure 1G) and did not return to the apical cortex to mature (Figure 1C). We conclude that *bld10's* centrosome asymmetry defect is not due to aberrant centriole migration.

To get mechanistic insight into *bld10*'s phenotype, we used live imaging to measure the dynamic localization of three GFP-tagged pericentriolar matrix (PCM) markers: γ-tubulin (γ-Tub), Mini spindles (Msps; CKAP5 in vertebrates) and centrosomin (Cnn; CDK5Rap2 in vertebrates) [17–19]. Wild-type neuroblasts showed robust localization of γ-Tub, Msps, and Cnn to the apical centrosome during interphase. After centrosome splitting, all three PCM markers were downregulated from the basal centrosome (shedding phase) but reaccumulated during prophase (maturation phase; Figure 2A and Movie S1). *bld10* mutant neuroblasts also correctly localized γ-Tub, Msps, and Cnn to the apical interphase centrosome. However, similar to the MTOC marker Jupiter, γ-Tub, Msps, and Cnn were not downregulated from the separating centriole (Figure 2B and

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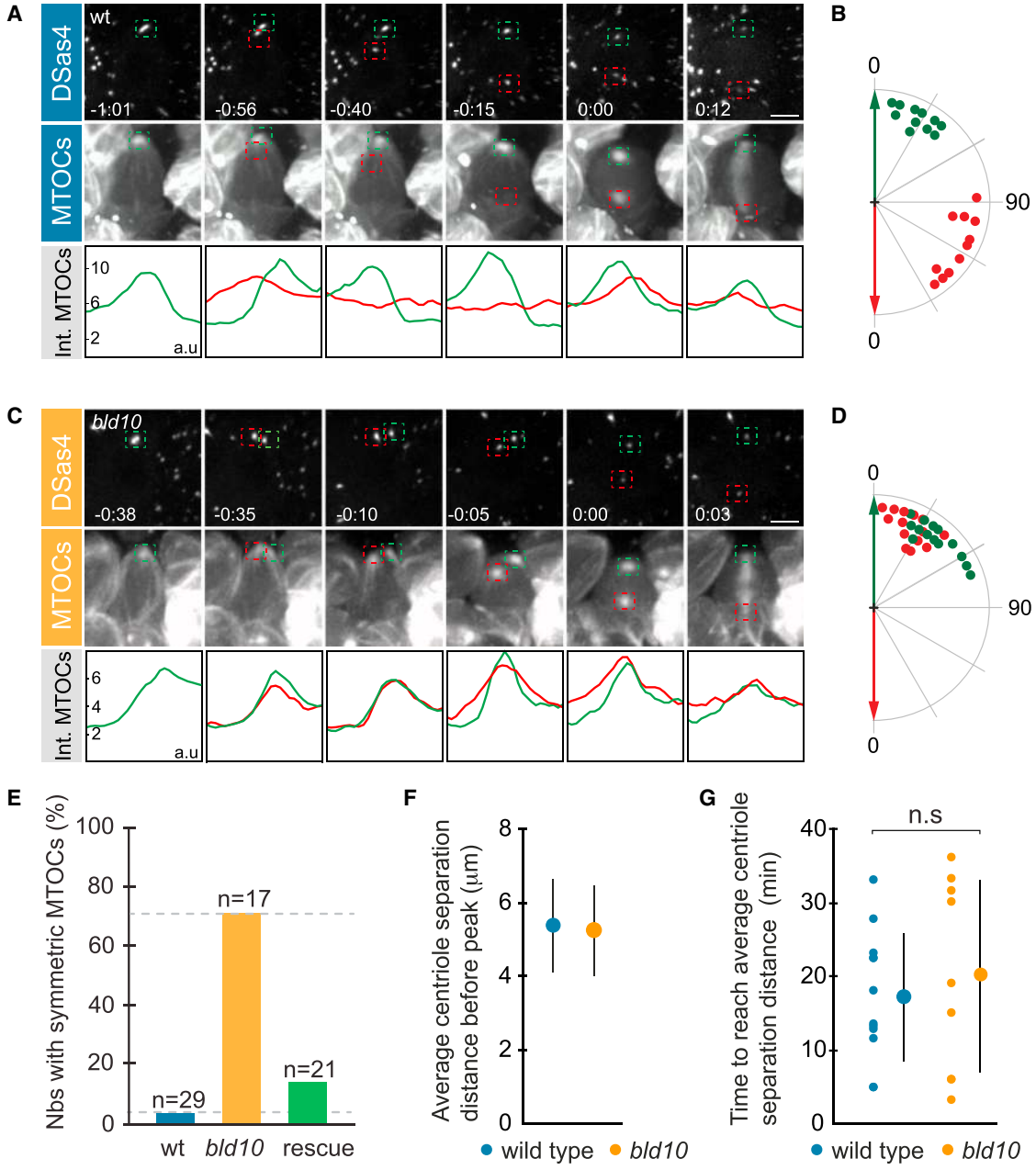

Figure 1. Bld10 Is Required for Centrosome Asymmetry

(A and C) Wild-type (A) and *bld10* mutant (C) third-instar larval neuroblasts expressing the centriolar marker DSas4::GFP (top row) and the MTOC marker Cherry::Jupiter (middle row). The green and red lines below the image sequences represent Cherry::Jupiter intensity values of the apical (green box) and basal (red box) centrosomes, respectively.

(B and D) Radial centrosome distribution plot of wild-type (B) and *bld10* mutants (D) depicting the maximal deviation of the apical (green) and basal (red) MTOC in relation to the cell division axis, denoted with the 0° line. Green and red arrows highlight the apical (green)-basal (red) polarity and division axis.

(E) Quantification of centrosome asymmetry phenotype in wild-type, *bld10* mutant, and rescued (*bld10* mutants, expressing bld10::GFP) neuroblasts expressing Cherry::Jupiter only.

(F) Average centriolar distance just before centriole separation reaches a maximum. Error bar indicate the SD.

(G) Time to reach average centriolar distance. The difference is statistically not significant (n.s.; p = 0.5748).

Int., intensity; a.u., arbitrary units; MTOC, microtubule-organizing center. Time is shown as hr:min. Scale bar, 5 μm. See also Figure S1.

Movie S2). We measured centrosome size and plotted a centrosome asymmetry index (see the Supplemental Experimental Procedures), starting at centrosome splitting until metaphase. Wild-type centrosomes developed a clear size asymmetry during the shedding phase and reduced it during the maturation phase (Figure 2C). *bld10* mutant centrosomes stayed similar in size, manifested in an asymmetry index below 1.5 (Figure 2D). Centrosome size and intensity measurements also revealed that in most wild-type neuroblasts, γ-Tub, Msps, and Cnn were removed from the basal centrosome ~15 min after centrosome splitting (Figure 2E). Basal wild-type centrosomes were essentially devoid of γ-Tub after that time,

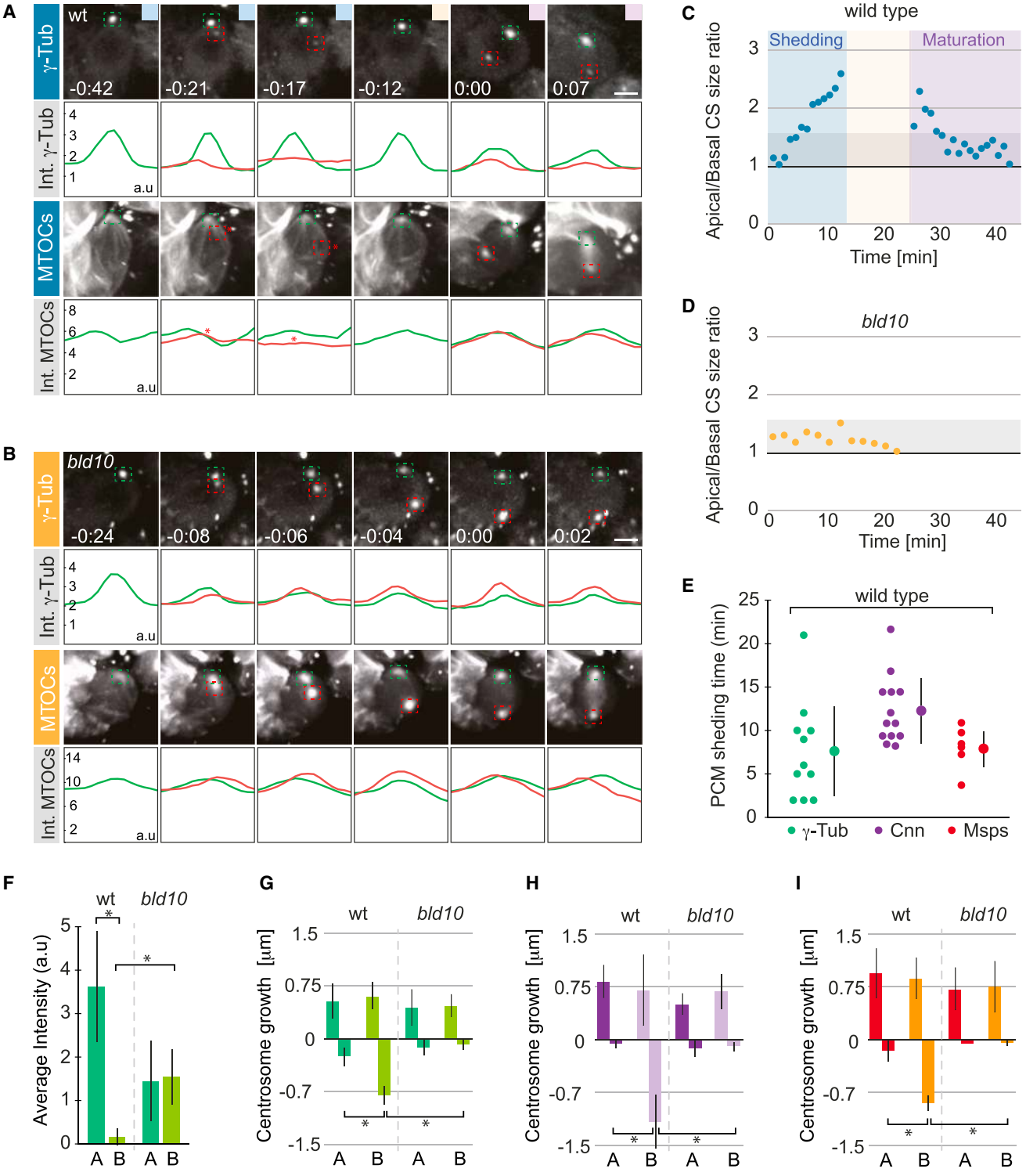

Figure 2. *bld10* Mutant Centrosomes Are Able to Mature but Fail to Downregulate PCM

(A and B) Wild-type (A) and *bld10* mutant (B) neuroblasts expressing the pericentriolar marker γ-tub::GFP and the MTOC marker Cherry::Jupiter. Colored insert boxes refer to the shedding phase (light blue), the maturation phase (light pink), and the phase in between (light yellow). Since the basal centrosome sheds almost all PCM, we cannot reliably measure its size once shedding is completed (light yellow). In all panels, the green and red dashed boxes label the apical daughter and the basal mother centrosome (CS), respectively. Asterisks denote intensity measurements with uncertainty (green line, apical CS; red line, basal CS).

(C and D) Asymmetry index graph (size of apical or bigger CS divided by the basal or smaller CS) of a representative wild-type (C) or *bld10* mutant (D) neuroblast.

(E) Measured PCM shedding time for γ-tub::GFP (green), Cnn::GFP (purple), and Msps::GFP (red) in wild-type neuroblasts. Approximately 15 min after centrosomes split, PCM markers are shed from the basal centrosome. Mean and SD are shown next to individual measurement points.

(legend continued on next page)

whereas *bld10* mutants contained equal amounts of this PCM marker (Figure 2F). We further compared changes in centrosome size and found that wild-type apical centrosomes predominantly grew, whereas basal centrosomes increased (maturation phase) and decreased (shedding phase) their size to almost the same extent. *bld10* mutant centrosomes were able to enlarge but showed very little size reduction, comparable to apical wild-type centrosomes (Figures 2G–2I). We conclude that *bld10* mutant centrosomes are able to mature but fail to downregulate the PCM markers γ-Tub, Msps, and Cnn.

The results above suggest two possible mechanisms for centrosome asymmetry: (1) Bld10 could prevent premature mother centrosome maturation by blocking the precocious accumulation of PCM proteins. (2) Alternatively, Bld10 could promote PCM shedding right after centrosomes separate, thereby preventing the basal mother centrosome to prematurely become an MTOC. We devised an in vivo pulse-chase labeling experiment to distinguish between these two possibilities. To this end, we tagged Cnn at its endogenous locus with the photoconvertable fluorescent protein mDendra2 [20] (see the Supplemental Experimental Procedures and [21]). If Bld10 blocks premature PCM accumulation, mother centrioles should quickly shed photoconverted Cnn and prematurely reaccumulate unconverted Cnn in *bld10* mutants. Vice versa, if PCM shedding is compromised, we should be able to follow the photoconverted centrosomes from the moment they separate until telophase. We found that apical wild-type daughter centrioles retained the majority of photoconverted Cnn::mDendra2 from early interphase until prophase (possibly longer), indicating that very little Cnn protein gets exchanged. The basal mother centriole, however, lost photoconverted Cnn::mDendra2 within approximately 10–15 min after centriole separation (n = 5), confirming that Cnn is shed quickly (Figure 3A and Movie S3). Interestingly, *bld10* centrioles retained photoconverted Cnn::mDendra2 for at least 45 min after separation (n = 7). In some cases, one of the centrioles decorated with photoconverted Cnn::mDendra2 was even inherited by the newly formed GMC (Figure 3B and Movie S4). We conclude that (1) on the apical centrosome, Cnn protein turnover is absent or significantly reduced during interphase, that (2) on the basal centrosome, Cnn is shed quickly and replaced with new Cnn when maturation sets in, and that (3) *bld10*'s centrosome asymmetry defect is not due to premature centrosome maturation. Instead, separating basal centrioles fail to shed Cnn in particular and possibly PCM proteins in general.

To elucidate the molecular mechanism underlying PCM shedding, we first analyzed the relationship between Bld10, Centrobin (Cnb), and Pericentrin (PCNT)-like protein (Plp). Recently, it was shown that Cnb is necessary and sufficient for PCM retention on the apical daughter neuroblast centrosome [8]. However, gain- and loss-of-function experiments with Cnb did not perturb Bld10's localization (Figures S2A–S2C). Similarly, as in the wild-type [7], Cnb was localized asymmetrically in *bld10* mutants (Figures 4A and 4D). *plp* mutants fail to downregulate γ-Tub on the mother centrosome [9]. We found that in *plp* mutant neuroblasts, the basal centrosome retained Cnn and MTOC activity during interphase (Figures S3A and S3B). Interestingly, photoconversion experiments showed that similar to *bld10*, Cnn shedding from the basal centrosome was compromised in *plp* mutants (n = 4) (Figure 3C). However, Plp's localization is not perturbed in *bld10* mutant neuroblasts (Figures S2D–S2G), and Bld10 was normally localized in *plp* mutants (Figures S2H–S2J). Knockdown of *plp* in *bld10* mutants (see the Supplemental Experimental Procedures) did not enhance *bld10*'s PCM shedding phenotype, but due to the occurrence of additional phenotypes (fragmented or multiple centrosomes; data not shown), the shedding phenotype could also be partially masked (Figure S3C). In sum, we conclude that Bld10 is regulating centrosome asymmetry independently of Cnb and that Plp is also required to shed Cnn.

Since the mitotic kinase Polo has been implicated in PCM retention during interphase [8, 9] we assayed Polo localization dynamics in wild-type and *bld10* mutant neuroblasts. Recently, it was reported that Polo localizes to the apical centrosome during interphase and is only detectable at the basal centrosome during prophase, when maturation sets in [9]. We used a Polo::GFP protein trap line [22] and confirmed that Polo is stably localized to the apical interphase centrosome [6, 9]. Surprisingly, we found weak Polo also on the separating mother centrosome (Figure 3D; time frame −0:47). Subsequently, Polo disappeared from the basal mother centriole within 10 min (±4 min; n = 7), comparable to Cnn, γ-Tub, and Msps shedding times (Figures 3F and 2E). With a genomic Polo::GFP transgene [23], showing lower fluorescence intensity, Polo was found to be localized on both centrosomes in *bld10* mutants (Figure 3E). These data suggest that in wild-type neuroblasts, Polo is not just recruited onto the basal mother centrosome by prophase as previously reported [6, 9], but is also subject to shedding during interphase. Polo is required for PCM retention since in *bld10* mutant neuroblasts treated with the Polo inhibitor BI2536 (see the Supplemental Experimental Procedures and [8]), both centrosomes lose MTOC activity (Figure 3G). Thus, we conclude that shedding of Polo is a requirement for the subsequent shedding of Cnn, γ-Tub, and Msps, enabling basal mother centrosome dematuration and the establishment of centrosome asymmetry.

Finally, we analyzed the consequences of disrupted centrosome asymmetry. We labeled the daughter centriole with Cnb::YFP [7] and assayed centrosome segregation. We confirmed that wild-type neuroblasts faithfully retain the Cnb^{+} daughter centriole, whereas the Cnb^{-} centrosome segregates into the GMC (100%, n = 10; Figures 4A–4C) [7]. *bld10* mutants showed correct asymmetric Cnb localization, but ~45% (n = 11) of *bld10* mutant neuroblasts wrongly retained the mother centriole and segregated the daughter centriole into the GMC (Figures 4C–4E). Cnb^{+} centrosomes are usually bigger in wild-type and *bld10* mutant neuroblasts (Figure 4F), but centrosome segregation is independent of MTOC activity and size since *bld10* mutant neuroblasts often retained the smaller centrosome (Figure 4E). We conclude that centrosome asymmetry is required for faithful centrosome segregation.

(F) Apical and basal centrosome γ-tub::GFP (green) intensity, measured 15 min after centrosomes splitting. Mean and SD are shown next to individual measurement points.

(G–I) CS growth measurements for γ-tub::GFP (green; G), Cnn::GFP (purple; H), and Msps::GFP (red, I). Bar graphs show averaged values and standard deviation for wild-type and *bld10* mutants.

Asterisks in (F)–(I) indicate $p < 0.0001$. Int., intensity; a.u., arbitrary units; A, apical CS; B, basal CS; MTOC, microtubule-organizing center. Time is shown as hr:min. Scale bar, 5 μm. See also Movies S1 and S2.

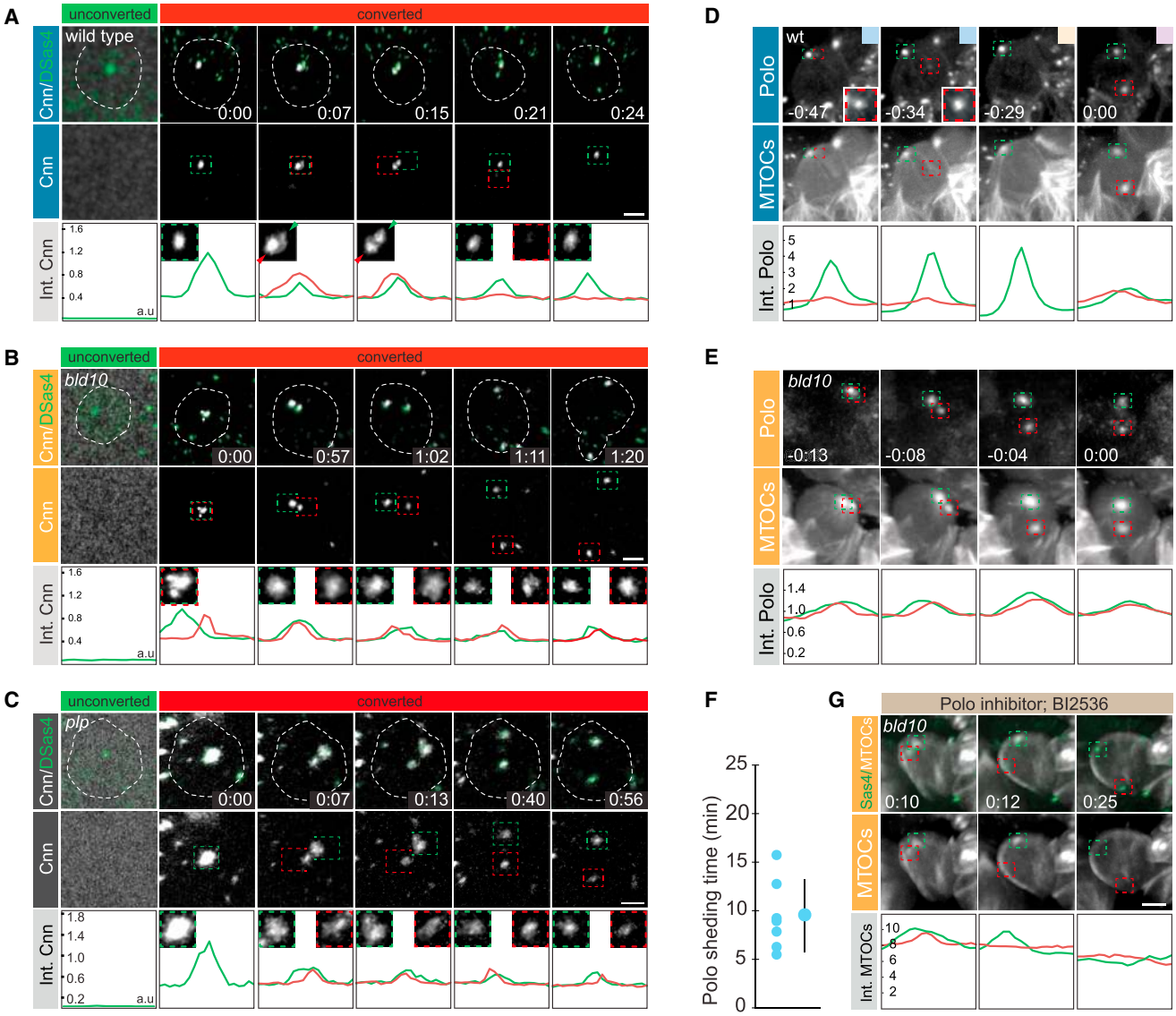

Figure 3. Neuroblasts Establish Centrosome Asymmetry through Shedding of Polo and PCM Proteins from the Mother Centrosome

The PCM marker Cnn, endogenously tagged with mDendra2 (white), was coexpressed with the centriolar marker Sas4::GFP (green) to facilitate centrosome tracking. Photoconversion was performed shortly after telophase, before centrosome separation occurred. Time point 0:00 refers to the first frame after photoconversion of Cnn::mDendra2.

(A–C) Image sequence of a wild-type (A), *bld10* mutant (B), and *plp* mutant (C) neuroblast. Wild-type basal centrosomes turn over photoconverted Cnn within 15–20 min. In *bld10* mutants, both centrosomes retain photoconverted Cnn until telophase. Basal centrosomes in *plp* mutants retain photoconverted Cnn for at least 1 hr. The white dashed line represents the cell outline. The lower panels show an intensity plot of converted Cnn::mDendra2 at apical (green dotted box) and basal (red dotted box) centrioles. Inserts show high-magnification pictures of photoconverted centrosomes.

(D) Polo::GFP (protein trap line [22]) expressed in wild-type neuroblasts. Inserts at time point −0:47 and −0:34 show higher magnifications of the basal centrosome with enhanced signal intensity. Colored insert boxes refer to the shedding phase (light blue), the maturation phase (light pink), and the phase in-between (light yellow). Polo intensity is plotted below.

(E) *bld10* mutant neuroblast expressing Polo::GFP transgene [23].

(F) Scatter plot showing the measured time to shed Polo in wild-type neuroblasts.

(G) *bld10* mutant neuroblast treated with the Polo inhibitor BI2536. Centrioles are labeled with Sas4::GFP (green). MTOCs are labeled with Cherry:Jupiter (white). MTOC intensity is plotted below.

MTOC, microtubule-organizing center; Int., intensity; a.u., arbitrary units. Time is shown as hr:min. Scale bar, 5 μm. See also Figures S2 and S3 and Movies S3 and S4.

Since *bld10* mutant neuroblasts have mispositioned MTOCs in relation to the apical-basal division axis (Figures 1B and 1D), we analyzed spindle orientation (see the Supplemental Experimental Procedures). Immunohistochemistry experiments showed that *bld10* mutant neuroblast spindles deviate from the regular orientation range, with isolated cases of extreme misalignment (Figures 4G and 4H). Metaphase spindles, aligned orthogonally to the apical-basal polarity axis, can induce symmetric neuroblast divisions, resulting in an increase of the neural stem cell pool [14]. However, neuroblast numbers were unchanged in *bld10* mutants compared to control brains (data not shown), and we did not find symmetric

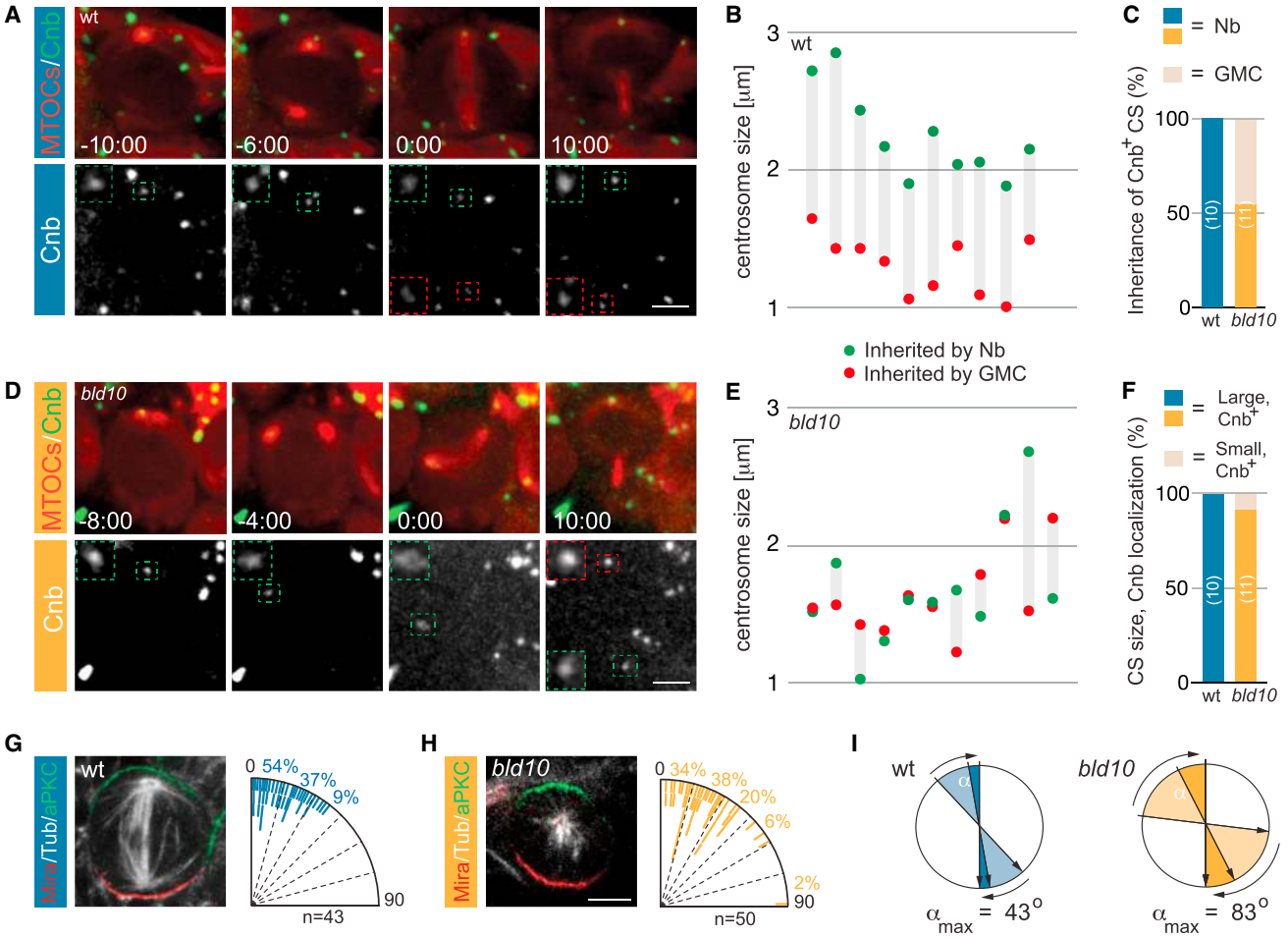

Figure 4. Centrosome Asymmetry Is Required for Correct Centrosome Segregation and Spindle Orientation

(A and D) Wild-type (A) and *bld10* mutant (D) neuroblasts expressing the daughter centriole specific marker Cnb::YFP (green) and the MTOC marker Cherry::Jupiter (red). Inserts show a high-magnification picture of the Cnb$^+$ centrioles. Note that at interphase and prophase, only the apical daughter centrosome contains a Cnb$^+$ centriole.

(B and E) Summary of wild-type (B) and *bld10* mutant (E) centrosome segregation pattern. Centrosome pairs (green and red dots, connected by gray line) of individual neuroblasts are displayed.

(C) Percentage of wild-type (blue) and *bld10* mutant (yellow) neuroblasts inheriting the Cnb$^+$ centrosome after cell division.

(F) Correlation between centrosome size and Cnb$^+$ localization.

(G and H) Representative picture of a wild-type (G) and *bld10* mutant (H) neuroblast stained with apical aPKC (green), α-tubulin (white) and basal Mira (red). Each tick mark (blue, wild-type; yellow, *bld10*) in the graph represents the orientation of the metaphase spindle with respect to the polarity axis for individual neuroblasts.

(I) Spindle correction angles are plotted for both wild-type (blue) and *bld10* (yellow). Darker shading indicates the mean correction angle (α mean). Lighter shading represents the maximal correction angle (α max). Wild-type: α max = 43°, α mean = 10° ± 8.5°; n = 26. *bld10*: α max = 83°, α-mean: 27° ± 23°; n = 22.

Nb, neuroblast; GMC, ganglion mother cell. MTOC, microtubule-organizing center. Time in min:sec; Scale bar is 5 μm.

neuroblast divisions with live imaging. Instead, our time-lapse experiments showed that *bld10* mutant centrosomes prematurely formed misaligned bipolar spindles. Spindle rotation during metaphase corrected this misalignment (Figure 4I). Apical-basal polarity is a prerequisite for correct spindle orientation (reviewed in [24]), but apical and basal polarity markers localized normally in *bld10* mutants (data not shown). Similarly, the spindle orientation regulators, Partner of Inscuteable (Pins; LGN/AGS3 in vertebrates) and the NuMA ortholog Mud, were also correctly localized (data not shown). We conclude that controlled PCM shedding and maturation is required for correct centrosome positioning but backup mechanisms exist, correcting for misaligned metaphase spindles.

Many cell types, including stem and progenitor cells, contain asymmetric centrosomes and segregate them nonrandomly, suggesting a connection between centrosome asymmetry and cell fate [25]. How centrosome asymmetry is regulated is currently not understood, but centrosome dematuration is a critical step in establishing centrosome asymmetry [4–9]. We found that the centriolar protein Bld10/Cep135, known as a centriole duplication and elongation factor [26–32], is required to establish centrosome asymmetry. On the basis of our data, we propose that Plp and Bld10 induce Polo's removal from the mother centriole, triggering the shedding of PCM proteins such as Cnn, γ-Tub, and Msps. Polo has been reported to be closely associated with centrioles [23], ideally positioned to phosphorylate both centriolar and PCM proteins [33]. Thus, we propose that Polo-mediated phosphorylation of PCM proteins maintains a stable interaction between the centriole and surrounding PCM (Figures S3D and S3E).

How Polo localization is regulated is currently not known, and we did not detect a direct molecular interaction between Bld10 and Polo (data not shown). Although we do not find centriolar markers to be mislocalized in *bld10* mutants (at the resolution level of confocal microscopy), it is possible that structural centriole defects, as detected in *bld10* mutant spermatocytes and wing disc cells [10, 31, 34], could affect PCM turnover rates. However, since Bld10 is not asymmetrically localized (data not shown), it is difficult to conceive how such defects specifically compromise the behavior of the mother but not the daughter centrosome.

Although perturbed centrosome asymmetry does not seem to undermine neuroblast polarity, the cell cycle, or physical and molecular asymmetric cell division, we cannot exclude the possibility that centrosome asymmetry could have long-term consequences currently beyond our ability to detect. Interestingly, defects in centrosome maturation or mutations in Cep135 can cause neurodevelopmental disorders such as primary microcephaly [2, 35–37]. It will be interesting to address the question whether lack of Cep135 is causing microcephaly due to compromised centrosome asymmetry and dematuration.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and four movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.05.050.

Author Contributions

P.S. and C.C. conceived and designed the project. P.S. performed the experiments with help from A.R.N. P.S., A.R.N., and C.C. analyzed the data. P.S. and C.C. wrote the manuscript.

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