Supplementary material

The SCF ubiquitin ligase protein Slimb regulates centrosome duplication in *Drosophila*

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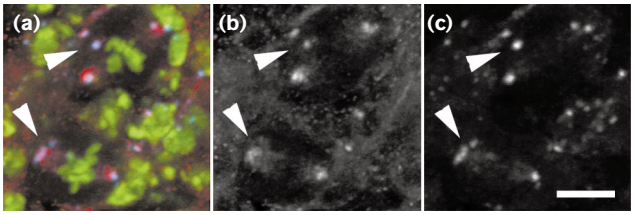
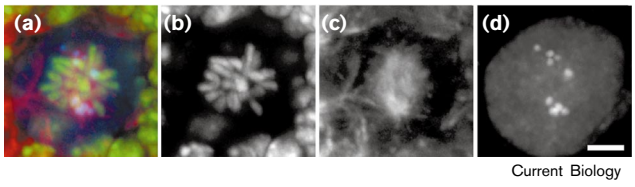
Figure S1



Off-axis centrosomes in the *slimb*^{crd} mutant contain ASP. **(a)** Adjacent metaphase *slimb*^{crd} mutant neuroblasts (arrowheads) triple-labeled for DNA (green), γ-tubulin (blue) and ASP (red). The ASP channel from (a) is shown alone in **(b)**. The lower arrowhead points to the ASP signal at a cluster of off-axis centrosomes; the upper arrowhead points to an off-axis extra centrosome. **(c)** The γ-tubulin channel from (a). The scale bar represents 5 μm.

Figure S2



Polyploid *slimb*^{crd} neuroblasts can be seen with bipolar spindles and excess centrosomes. **(a)** Merged Z-series of an aberrant *slimb*^{crd} mutant polyploid neuroblast fluorescently labeled for DNA (green), tubulin (red) and CP190 (blue). **(b–d)** The DNA, tubulin and centrosome channels, respectively, from (a). The scale bar represents 5 μm.

Supplementary materials and methods

Fly stocks

Animals that are trans-heterozygous for *slimb*^{crd} and a previously identified recessive lethal *slimb* allele, *slimb* ^{PZ00295} [S1], failed to survive beyond mid-larval development. Cells in the central nervous system of these larvae displayed a mitotic phenotype indistinguishable from that of *slimb*^{crd} hemizygotes (not shown). The *slimb*^{crd} homozygotes die at the larval–pupal boundary; animals hemizygous for *slimb*^{crd} exhibited lethality at an earlier stage of the third-larval instar, suggesting that the *P* allele is hypomorphic. Furthermore, the *slimb* ^{PZ00295} mutation was both viable and fertile in combination with the *slimb*^{crd} revertant chromosomes. Amorphic *slimb* alleles have previously been shown to result in embryonic lethality [S1].

Immunofluorescence and confocal microscopy

Whole-mount larval brains were prepared for immunofluorescence as described [S2]. Triple-labeled specimens were generated by using combinations of the following antibodies: anti-γ-tubulin (Sigma, DM1A at 1:200); anti-CP190 (at 1:500; [S3]); anti-CNN (at 1:500 [S4]); and anti-ASP (at 1:200, [S5]). The fluorescent DNA stains ToPro-3 or Sytox Green (both at 1:2000; Molecular Probes) were interchangeably used as required. Fluorescently conjugated secondary antibodies used were anti-mouse Texas Red conjugate (Jackson Immunoresearch Labs) and an anti-rabbit Alexa-488 conjugate (Molecular Probes). Z-series data were collected on a Bio Rad MRC 1024 confocal system. Sequential image acquisition prevented bleed-through artifacts from both double- and triple-labeled samples. Projections of Z-series were created with NIH image, and final images adjusted for printing using Adobe PhotoShop.

Labeling of S-phase cells in larval brains

We followed the procedure of Mills *et al.* [S6] with the following modifications. Wild-type and mutant brains were isolated intact and carried through the entire protocol as whole mounts. Both wild-type and mutant brains from each replicate experiment were performed simultaneously and in the same buffers. Replicating DNA was labeled *in situ* with Alexa 488 dUTP (Molecular probes). Samples from several independent experiments were analyzed by confocal microscopy as above.

Supplementary references

S1. Theodosiou NA, Zhang S, Wang WY, Xu T: **Slimb coordinates wg and dpp expression in the dorsal-ventral and anterior–posterior axes during limb development.** *Development* 1998 **125:**3411-3416.

S2. Robinson JT, Wojcik EJ, Sanders MA, McGrail M, Hays TS: **Cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in *Drosophila*.** *J Cell Biol* 1999, **146:**597-608.

S3. Whitfield WG, Millar SE, Saumweber H, Frasch M, Glover DM: **Cloning of a gene encoding an antigen associated with the centrosome in *Drosophila*.** *J Cell Sci* 1988, **89:**467-480.

S4. Li K, Kaufman TC: **The homeotic target gene centrosomin encodes an essential centrosomal component.** *Cell* 1996, **85:**585-596.

S5. do Carmo Avides M, Glover DM: **Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers.** *Science* 1999, **283:**1733-1735.

S6. Mills AD, Coleman N, Morris LS, laskey RA: **Detection of S-phase cells in tissue sections by in situ DNA replication.** *Nat Cell Biol* 2000, **2:**244-245.