A perinuclear microtubule-organizing centre controls
nuclear positioning and basement membrane secretion

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Non-centrosomal microtubule-organizing centres (ncMTOCs) have a variety of roles that are presumed to serve the diverse functions of the range of cell types in which they are found. ncMTOCs are diverse in their composition, subcellular localization and function. Here we report a perinuclear MTOC in Drosophila fat body cells that is anchored by the Nesprin homologue Msp300 at the cytoplasmic surface of the nucleus. Msp300 recruits the microtubule minus-end protein Patronin, a calmodulin-regulated spectrin-associated protein (CAMSAP) homologue, which functions redundantly with Ninein to further recruit the microtubule polymerase Msp—a member of the XMAP215 family—to assemble non-centrosomal microtubules and does so independently of the widespread microtubule nucleation factor γ-Tubulin. Functionally, the fat body ncMTOC and the radial microtubule arrays that it organizes are essential for nuclear positioning and for secretion of basement membrane components via retrograde dynein-dependent endosomal trafficking that restricts plasma membrane growth. Together, this study identifies a perinuclear ncMTOC with unique architecture that regulates microtubules, serving vital functions.

Microtubule (MT) organization supports critical cellular functions in cell division, cell polarity and intracellular trafficking. The best-known microtubule-organizing centre (MTOC) in animal cells is the centrosome. However, various cell types across species lack a functional centrosome after exit from the cell cycle and following differentiation. In these cases, ncMTOCs function as alternative sites to accommodate the organization of MT networks specialized for differentiated cell types1–4. Whereas knowledge of the centrosome is extensive, the roles of ncMTOCs across species lack a functional centrosome after exit from the cell cycle and following differentiation. In these cases, ncMTOCs serve the unique needs of diverse cell types, it is essential to determine how ncMTOCs are assembled and identify the key effectors of MT assembly.

Here we report the discovery of an ncMTOC that is assembled on the surface of nuclei in Drosophila larval fat body cells, a differentiated cell type that has critical secretory functions and serves the metabolic needs of the organism. This study identifies a perinuclear ncMTOC with unique MT assembly mechanisms that controls physiological roles of fat body cells by supporting nuclear positioning and vital secretory functions.

Results

A perinuclear MTOC is assembled in fat body cells. Postmitotic polyplloid cell types in Drosophila, such as salivary gland, midgut and malpighian tubule cells, lack centrosomes. We found that centrosomes are also lost in polyplloid fat body cells (Fig. 1a), which are functionally similar to vertebrate liver and adipose cells, having high metabolic and secretory activities.

Fat body cells have a prominent perinuclear organization of MTs. MTs are highly enriched circumferentially at the nuclear surface and also radiate outward towards the plasma membrane (Fig. 1a). Consistent with this being an MTOC, the centrosomal protein Centrosomin (Cnn) and MTs are positioned on the cytoplasmic face of the nuclear envelope (Fig. 1b). Electron microscopy imaging confirmed the presence of MTs oriented circumferentially and perpendicularly (radially) to the nuclear surface (Fig. 1c and Extended Data Fig. 1a). Additionally, localization of a Nod–β-galactosidase fusion protein shows that MT minus ends are enriched at the MTOC on the nuclear surface (Fig. 1d). Notably, MT regrowth experiments show that the nuclear surface is the primary site for MT assembly (Fig. 1e).

Cold treatment, which typically causes MT polymers to disassemble, did not overtly affect the organization of the MT array at the nuclear surface (Extended Data Fig. 1b), indicating that these MTs are highly stable. Consistent with this, fat body MTs are acetylated

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Together, these data indicate the presence of a ncMTOC that organizes stable MTs at the nuclear surface of fat body cells.

Fat body microtubules, but not actin, are essential for nuclear positioning. To investigate the function of the MT array in fat body cells we disrupted MTs directly by either knocking down expression of the MT subunits (α-Tubulin or β-Tubulin) or overexpressing poly glutamylated (Extended Data Fig. 1c,d); these post-translational modifications of tubulin are associated with stabilized MTs20.

**Fig. 1 | Fat body cells assemble a perinuclear ncMTOC.** a, Top: immunofluorescent staining of fixed fat body tissues from third-instar larvae labelled to reveal MTs (α-Tubulin (α-Tub)), the centrosomal protein Cnn, nuclear Lamin C and DNA (DAPI). Bottom: magnified view of a fat body cell. b, A close-up view of the nuclear surface showing MTs, Cnn, Lamin C and nuclear DNA. c, Electron microscopy image of fat body MTs (three stitched images). MTs are coloured in green and nuclei are tinted blue. Two general populations of radial MTs are evident; those anchored at the nuclear surface, presumably by their minus ends (arrows) and another group that appear to branch from the circumferential MTs (arrowheads). See also Extended Data Fig. 1a. d, Localization of the Nod–β-galactosidase (Nod–β-gal) fusion protein at the nuclear surface marks the MT minus ends. e, MT regrowth in fat body cells 5 min after recovery from vinblastine treatment shows MT assembly enriched at the perinuclear ncMTOC. Insets show magnification of a single fat body cell; arrows point to sites of MT regrowth. Genotype details are in Supplementary Tables 2 and 3. Experiments were performed ten times in a,b, once in c and twice in d,e.
the MT-severing enzymes Spastin or Katanin-60. Knockdown of either tubulin subunit markedly disrupted MTs (Fig. 2a,c) and the expression and stability of the other subunit; that is, knockdown of α-Tubulin resulted in reduced β-Tubulin expression and vice versa (Fig. 2b,d). Overexpression of Spastin or Katanin-60 also disrupted fat body MTs (Fig. 2e–h).

In each fat body cell, the nucleus resides in the geometric centre (centroid) (Figs. 2i and 3a). When fat body MTs were disrupted by either approach, nuclear positioning was altered (Fig. 2i). In contrast to MT disruption, blocking actin nucleation by Arp2 or Rh1 knockdown reduced F-actin signal but did not disrupt nuclear positioning (Fig. 2g). These data show that MTs, but not actin, are necessary for the proper positioning of nuclei in fat body cells.

Centrosomal proteins are localized at the fat body ncMTOC but are not required for MT assembly. We next determined the requirement of centrosomal proteins and MT regulatory proteins for fat body MTOC functions using nuclear positioning as a methodological readout (Supplementary Table 1 and Supplementary Video 1). We also surveyed the localization of centrosomal proteins and MT regulators at the perinuclear MTOC (Supplementary Table 1). This survey showed that the major core components of the pericentriolar material (PCM) were present at the fat body MTOC (with the exception of Spd-2), but core centriolar proteins were absent (Extended Data Fig. 2 and Supplementary Table 1).

However, no single centrosomal mutant that we tested disrupted fat body nuclear centricity or ncMTOC assembly (Fig. 3a, Supplementary Table 1 and Extended Data Fig. 3). Reasoning that there might be functional redundancy in MT assembly, as is the case with Cnn and Spd-2 at centrosomes21, we tested pairwise combinations of mutants or knockdowns using RNA interference (RNAi), but found no combination of centrosomal proteins that was required for the fat body ncMTOC to assemble MTs or affect nuclear positioning (Fig. 3a and Extended Data Fig. 3). These data indicate that although the fat body ncMTOC shares a majority of the core centrosome PCM components, they may not be required for MTOC function.

γ-Tubulin is not required for MT assembly at the fat body ncMTOC. γ-Tubulin, partnering with γ-Tubulin complex proteins (GCPs) to form the γ-Tubulin ring complex (γ-TuRC), is widely employed as a MT nucleator or anchor at centrosomes and other MTOCs1–3,5,6,9,10,22. Compared with larval brains, fat bodies express about 120-fold lower levels of γ-Tubulin (Extended Data Fig. 4a). Nevertheless, its localization is detected at the ncMTOC throughout larval stages (Fig. 3b and Extended Data Fig. 4d), together with other γ-TuRC components, including GCP2 and GCP3 (Extended Data Fig. 2).

To investigate the requirement for γ-Tubulin at the fat body ncMTOC, we examined a null mutant and three independent RNAi lines, all of which showed depletion by western blotting and/or no detectable γ-Tubulin at the nuclear surface in the larval fat body throughout developmental stages (Extended Data Fig. 4a–d). The lack of detectable signal in first-instar larvae indicates that the mutant effectively eliminated expression, and that there is no substantial residual maternal supply in fat body cells before the third-instar larval stage, when most of our experiments were performed. Surprisingly, we found that fat body cells lacking γ-Tubulin (γTub23C) or GCP3 (grip91) had normal nuclear positioning (Fig. 3a,c and MT assembly (Fig. 4a–d and Extended Data Fig. 4e, f). Since fat body MTs are highly stabilized, a requirement for γ-Tubulin during early stages of fat body MTOC, when some residual maternal supply might persist, could be masked by MT stabilization in the γ-Tubulin mutant. To address this possibility, we performed MT regrowth and found that MT regrowth was indistinguishable between wild-type and γ-Tubulin-depleted fat body cells (Extended Data Fig. 4f), demonstrating that γ-Tubulin has no substantial role in MT assembly at the fat body ncMTOC.

At centrosomes, γ-Tubulin is the major regulator of MTs, whereas Aurora A kinase (AurA) independently regulates the remaining MTs24–26. In the fat body ncMTOC, however, core-depletion of γTub23C and aurA had no effect on nuclear positioning or MT assembly (Fig. 3a), consistent with a similar lack of requirement for these MT regulators at the apical ncMTOC in Caenorhabditis elegans embryonic intestine21. Therefore, the widespread MT nucleator γ-Tubulin is not required for MT assembly at the fat body ncMTOC (Fig. 4j).

Msps controls fat body radial MT assembly independently of conventional partners. Among the 39 candidates tested in our survey (Fig. 3a), minisprindles (msps) knockdown (Extended Data Fig. 5a) uniquely impaired nuclear centricity (Fig. 3a,c). Msps functions at the growing MT plus end as a processive MT polymerase27. Msps localizes to the fat body ncMTOC (Fig. 3b), and msp knockdown significantly impaired radial MT assembly, whereas the dense circumferential MTs at the nuclear surface appeared unaffected (Fig. 4a–d), indicating that MT elongation is specifically impaired.

Transforming acidic coiled coil (TACC) is a binding partner of Msps, and its localization at centrosomes is regulated by AurA28–30. In oocytes, Msps is transported to spindle poles by the kinesin-14 motor Ncd31–33. However, these components are not required for ncMTOC assembly or nuclear centricity in the fat body (Extended Data Fig. 5b), indicating a distinct mechanism for Msps deployment at the fat body ncMTOC.

It was recently shown that Msps homologs function as MT nucleators by directly binding to and cooperating with γ-Tubulin complexes34–41. However, the role of Msps at the fat body ncMTOC is clearly distinct from these other contexts because the γ-TuRC is not required for the fat body ncMTOC. Previous work supports a role for Msps in MT growth independent of γ-Tubulin in interphase S2 cells31.

Together, these data show that Msps is required for MT assembly at the fat body ncMTOC, specifically for radial MT elongation, independently of previously described mechanisms.

Patronin controls circumferential MT assembly at the ncMTOC. Patronin/CAMSAP family proteins are MT minus-end proteins that stabilize MTs and have emerged as critical factors at ncMTOCs in a variety of organisms and cell types32. Patronin localized at the fat body ncMTOC (Fig. 3b); however, Patronin knockdown (Extended Data Fig. 6a) had no significant impact on nuclear centricity (Fig. 3a,c). Notably, Patronin knockdown significantly reduced the circumferential MTs but not the radial MTs (Fig. 4a–d), indicating that Patronin is involved in stabilization or assembly of the MTs most proximal to the MTOC.

Patronin/CAMSAP proteins stabilize MT minus ends and antagonize the activity of Kinesin-13 family depolymerases12–14. However, this antagonism does not prevail at the fat body ncMTOC because Kinesin-13 knockdown (Klp10A, Klp59C or Klp59D), did not suppress the reduced circumferential MTs in Patronin knockdown cells (Extended Data Fig. 6b).

Patronin/CAMSAP proteins associate with the MT-severing enzyme katanin35–38, an association proposed to remodel MTs and perhaps amplify MT minus ends39,40. However, in fat body cells, loss of Katanin p60 (Kat60), Katanin p60-like 1 (Kat-60L1), the regulatory subunit p80 (Kat80) or the other severing enzyme Spastin did not overtly impact MT assembly or nuclear positioning, and neither did the co-depletion of Kat80 and Spastin (Fig. 3a and Extended Data Fig. 6c,d). Disruption of another MT regulator, the Augmin (HAUS in mammals) complex32–41, with a dgt4 mutant also had no effect on the centroid nucleus or MT assembly in fat body cells (Extended Data Fig. 6e). These two potential MT amplification
Fig. 2 | Microtubule disruption, but not actin disruption impairs nuclear positioning. a–i. Fat body MTs were disrupted using two approaches, both involving the generation of genetically mosaic ‘flipout’ clones. In the first approach, expression of the genes encoding the MT subunits α-Tubulin and β-Tubulin was knocked down by RNAi. In the second approach, the MT-severing enzymes Spastin or Katanin-60 were overexpressed to disrupt MTs. The flipout clones were marked with green fluorescent protein (GFP) expression adjacent to the control cells, enabling comparison of knockdown and overexpression cell phenotypes side-by-side with control cells. Arrowheads indicate nuclei that have lost centricity. a,b. Knockdown of αTub84B reduces expression of α-Tubulin (a) and also β-Tubulin (b). c,d. Similarly, knockdown of βTub56D blocks expression or stability of both tubulin subunits. Overexpression of MT-severing enzymes Katanin-60 (e,f) or Spastin (g,h) in GFP-marked clones also results in MT disruption. i. All of these MT disruptions result in a loss of nuclear centricity in fat body cells, indicated with arrowheads in a–h. j. Disruption of actin assembly by knockdown of Arp2, an actin nucleator, or Rho1, a GTPase critical for actin assembly, did not impair nuclear positioning. Insets show the impaired assembly of cortical F-actin in Arp2 or Rho1 RNAi cells compared with the control cell. Details of genotype are in Supplementary Table 3. Fat body clonal analysis in (a–j) were performed twice with similar results. Quantification of nuclear positioning is shown in Fig. 3a.
Fig. 3 | Msp300, Shot, Msp5, Patronin and Nin but not γ-Tubulin are required for the fat body ncMTOC. a, The indicated mutants or RNAi-mediated knockdowns were surveyed for their requirement to position the nucleus at the centroid relative to cortical F-actin or membrane-anchored myristoylation domain of Src fused to red fluorescent protein (myr–RFP), both of which mark the cell boundary. Nuclear positioning in the indicated genotypes is quantified in the graph. Genes labelled in red are required for normal nuclear positioning. Data are shown as mean ± s.e.m. Statistics by two-tailed Student’s t-test. Number of independent clones or cells (n) analysed and other statistical details are shown in Source Data. OE, overexpression; DN, dominant negative. b, Images of fat body cells stained for Cnn in red and counterstained in green for the indicated proteins using either antibody staining or expression from a fluorescent protein-tagged transgene as indicated. GFP antibody was applied for GFP-tagged transgenes to enhance signal. See also Extended Data Fig. 2. c, The indicated genes were knocked down by RNAi in GFP-marked clones. Top: fat bodies containing control and GFP-marked knockdown clones were co-stained for DNA (Hoechst) and actin (CF568-Phalloidin) to assess nuclear centrity. Bottom: illustrations depicting the nuclear positioning in the indicated RNAi clones. Arrowheads indicate a loss of nuclear centrity. RNAi lines used here are msp5hM01906, γTub23ChL01171, PatroninhM01547 and NinhhMJ23837 (see RNAi validation in Extended Data Figs. 4b,c, 5a and 6a,g,h; see phenotype verification with other independent RNAi lines in Supplementary Table 4). Genotype details are in Supplementary Table 3. Staining experiments in b,c were performed three times with similar results.
Fig. 4 | Patronin and Ninein cooperatively assemble perinuclear MTs and recruit Msps for MT elongation independently of γ-Tubulin. **a**, Mosaic fat bodies were stained for α-Tubulin to assess MT assembly defects at the ncMTOC in control and GFP-marked RNAi cells. Middle and bottom panels show close-ups of the MT staining in the control (red box) and RNAi cells (green box). Circumferential MTs (arrowheads) and radial MTs (arrows) are indicated in a control cell. This experiment was performed three times with similar results. **b**, Diagrams depicting circumferential and radial MT assembly phenotypes in fat body cells from wild type and the indicated RNAi. **c-d**, Quantification of mean fluorescent intensity of radial microtubules (c) and circumferential microtubules (d) in the indicated RNAi clones compared with control cells. Msps RNAi versus control, *P* = 0.002 (radial; n = 6 control, n = 4 RNAI); γ-Tub23C RNAi versus control, *P* = 0.9316 (radial; n = 6 control, n = 4 RNAI); Patronin RNAi versus control, *P* = 0.1845 (radial; n = 8 control, n = 5 RNAI); Patronin RNAi + Nin RNAi versus control, *P* = 0.002 (radial; n = 6 control, n = 9 RNAI), *P* = 0.0004 (circumferential; n = 8 control, n = 5 RNAI). **e-g**, Msps localization at the nuclear surface (arrows) in the indicated RNAi clones marked with His–RFP. β. Quantification of Msps perinuclear intensity shown in e–g. Patronin + Nin RNAi versus control, *P* = 0.00007 (n = 4 control, n = 7 RNAI), Patentin RNAi + Nin RNAi versus control, *P* = 0.0213 (radial; n = 9 control, n = 6 RNAI), *P* = 0.0004 (circumferential; n = 8 control, n = 5 RNAI). **i**, Haemagglutinin (HA)–Msps (anti-HA) immunoprecipitation (IP) with endogenous Patronin in Drosophila S2 cells. Input was 2% of total lysate. Full blot is shown in Source Data. The co-immunoprecipitation experiment was repeated twice with consistent results. **j**, Working mechanism in fat body ncMTOC. Depiction of a fat body cell with MTs (green) emanating from the nucleus (N) and conceptual zoom-in showing Patronin and Ninein recruiting Msps to the ncMTOC to control radial MT assembly. Data in **c,d,h** are mean ± s.e.m; two-tailed Student’s *t*-test; *n* is the number of independent clones analysed; statistical details in Source Data. NS, not significant.
mechanisms appear to be not required for MT assembly at the fat body ncMTOC.

In sum, these results show that the ncMTOC regulator Patronin/CAMSAP is partially responsible for MT assembly or stability at the fat body MTOC, but does not function via the known mechanisms established in other contexts.

**Patronin cooperates with Ninein to organize the fat body ncMTOC by recruiting Msp3.** We reasoned that Patronin might work in conjunction with other minus-end proteins to assemble the fat body ncMTOC. We investigated γ-Tubulin and Ninein (Nin), two other MT minus-end proteins. CAMSAP and γ-Tubulin are proposed to act sequentially in the generation of non-centrosomal MTs in neurons where γ-Tubulin initiates MT nucleation and CAMSAP stabilizes MTs. In *C. elegans* larval epidermis, Patronin works in parallel with γ-Tubulin at ncMTOCs. In *Drosophila* fat body cells, however, co-depletion of Patronin and γ-Tubulin did not enhance the MT-organization phenotype of Patronin single knockdown (Extended Data Fig. 6f), indicating that γ-Tubulin does not function redundantly or cooperatively with Patronin at the fat body ncMTOC.

Nin is a MT-binding protein with anchoring function at the centriole subdistal appendages in mammals, but Nin also has ncMTOC roles in mammals, *Drosophila* and *C. elegans*. Nin localizes to *Drosophila* muscle and wing epithelial ncMTOCs and to the oocyte ncMTOC. Nin also localized to the fat body ncMTOC (Fig. 3b). Flies and mice with mutations in *ninein* are viable, and Nin mutants or knockdowns (Extended Data Fig. 6g,h) showed no overt effects on nuclear positioning (Fig. 3a,c), or MT assembly (Fig. 4a–d) in the fat body. However, knockdown of both Patronin and Nin disrupted nuclear positioning (Fig. 3a,c). Moreover, Patronin Nin double knockdown impaired both radial and circumferential MT assembly, whereas Patronin knockdown mostly affected circumferential MTs and Nin knockdown had no overt effects on its own (Fig. 4a–d). These data demonstrate that Patronin and Nin function redundantly in MT assembly at the fat body ncMTOC and indicates that each can effectively compensate for the other to control assembly of radial MTs.

Nin associates with γ-Tubulin, and in *C. elegans*, γ-Tubulin recruits NOCA-1 (a homologue of Nin) to the ncMTOC in epidermal cells, where they function together and in parallel with Patronin to regulate non-centrosomal MT assembly. However, at the fat body ncMTOC, γ-Tubulin did not recruit Nin (Extended Data Fig. 6i), and γ-Tubulin knockdown or null mutant did not enhance Patronin-knockdown phenotypes (Extended Data Fig. 6f). Moreover, Nin knockdown together with γ-Tubulin or gcp3 knockdowns had no effect on nuclear positioning in fat body cells (Extended Data Fig. 6j). Altogether, these findings show that Nin and Patronin cooperate in MT assembly at the ncMTOC without the functional involvement of γ-Tubulin.

We then examined whether Patronin and Ninein are required to recruit Msp3 to the ncMTOC. Knockdown of Patronin reduced Msp3 recruitment to the nuclear surface to 37.0% of control, whereas Nin knockdown had no obvious effect (Fig. 4e,f,h). Patronin Nin double knockdown, however, significantly diminished Msp3 localization to 19.6% relative to control (Fig. 4g,h). We used co-immunoprecipitation assays to show that Patronin associates with Msp3 (Fig. 4i). These combined data point to a mechanism for MT assembly at the ncMTOC in which Patronin and, perhaps also Nin, cooperate to recruit Mps3 to promote elongation of radial MTs independently of γ-Tubulin (Fig. 4j).

**The Nespin Msp300 anchors the fat body ncMTOC at the nuclear surface and recruits Shot and Patronin.** We investigated how the fat body ncMTOC is anchored to the nuclear surface, identifying the linker of nucleoskeleton and cytoskeleton (LINC) complex, comprising KASH domain-containing Nesprins and SUN domain-containing proteins that span both nuclear membranes as probable candidates for the anchoring protein. *Drosophila* has two Nesprins: Klarsicht (Klar) and Msp300, and one major SUN protein Klardo (Koi), all of which are localized to the nuclear envelope in fat body cells (Fig. 5a). Null alleles of *klar* or *koi* had little or moderate effect on nuclear centrality (Fig. 3a and Supplementary Table 1). However, knockdown or mutation of Msp300 disrupted nuclear centrity (Fig. 5b,c) and MT assembly (Fig. 5d), demonstrating that Msp300 is required for a functional ncMTOC.

Consistent with Msp300 controlling assembly of the ncMTOC, Patronin and Mps300 localization at the nuclear surface also required Msp300 (Fig. 5e,f). Additionally, Short stop (Shot), the only spectraplakin in *Drosophila*, was localized to the nuclear surface in an Msp300-dependent manner (Fig. 5g). Shot associates with Patronin and is required to localize Patronin to ncMTOCs in oocyte and ovarian follicle cells. Knockdown of *Patronin* did not affect Msp300 or Shot localization (Fig. 5h), consistent with a dependence of Patronin on Msp300 and/or Shot for perinuclear localization. These combined analyses suggest that Msp300 is the primary organizer of the perinuclear ncMTOC by recruiting Patronin with possible involvement of Shot. Patronin, together with Nin, generates circumferential MTs, and they also recruit Msps to assemble radial MTs (Fig. 5m).

**Shot depletion shifts the perinuclear ncMTOC to an ectopic MTOC.** While Shot localization to the perinuclear ncMTOC depends on Msp300 (Fig. 5g), Msp300 localization also depends on Shot. In contrast to Msp300 knockdown, knockdown of *shot* delocalized the perinuclear MTOC components, including Msp300, Patronin, and Mps300, to a centrosome-like MTOC focus in the cytoplasm (Fig. 5i and Extended Data Fig. 7a), whereas proteins not required for MTOC function, such as Cnn and γ-Tubulin, were not localized in this manner (Extended Data Fig. 7b–d). To test the idea that Msp300 may be the primary organizer of the ectopic MTOC induced by *shot* RNAi, we co-depleted Msp300 and *shot*. We found that Msp300 was critical for the *shot* RNAI-induced ectopic MTOC (Fig. 5j,k) and also for the recruitment of Patronin (Fig. 5j), suggesting that Msp300 may directly recruit Patronin. Co-depletion of *shot* with *Patronin* or *msps* also attenuated the ectopic MTOC (Fig. 5k), further indicating that Msp300, Patronin and Msps are key for MTOC function in the fat body. Together, these data indicate that Shot and Msp300 are co-dependent for their assembly into the perinuclear ncMTOC and that Msp300 may recruit Patronin and Msps independently of Shot (Fig. 5l).

Shot disruption generates additional pleiotropic phenotypes besides perturbing the perinuclear MTOC. Knockdown of *shot* reorganized F-actin into large aggregates that accumulated near the displaced MTOC (Extended Data Fig. 7e); deformed the nucleus (Extended Data Fig. 7f); reorganized organelles including the endoplasmic reticulum, Golgi and mitochondria (Extended Data Fig. 7g); and blocked secretion of basement membrane components (Extended Data Fig. 7h–i’), which accumulated near the ectopic MTOC (Extended Data Fig. 7i). These severe disruptions are in addition to the role of Shot at the ncMTOC, because *Msp300* knockdown blocked Shot localization to the ncMTOC but did not result in the severe and pleiotropic effects seen with *shot* knockdown.

**The fat body ncMTOC is essential for basement membrane secretion.** The fat body is the major source of synthesis and secretion of the collagen IV trimer, the subunits of which are encoded by the Collagen IV α1 (*Cg25c*) and α2 (*vkg*) genes (Fig. 6a). A small fraction of secreted Collagen IV is incorporated into fat body cell junctions, but the majority is secreted, transported via the haemolymph and deposited along with other basement membrane components, such as Perlecan (Trol), LanB1 and Nidogen (Ndg) at destination organs, such as imaginal discs and brain (Fig. 6a).
Fig. 5 | The Nesprin homologue Msp300 is required for MTOC assembly at the fat body nuclear surface and recruits Shot and Patronin. a, LINC complex components localized to the fat body nuclear periphery. b, c, Msp300 maintains nuclear centricity. Msp300 RNAi clones are marked with GFP (b) and mutant clones are marked by loss of GFP expression (c). RNAi or mutant clones are indicated with arrowheads and controls are indicated with arrows throughout the figure. d, Circumferential and radial MTs are diminished with Msp300 knockdown. e, Patronin localization to the nuclear surface is reduced in Msp300 RNAi clones (indicated by His–RFP). Quantification of Patronin perinuclear signal: 66.48 ± 10.21 (n = 10 control) versus 2.983 ± 0.7863 (n = 9 Msp300 RNAi clone), P = 0.000019. f, Msp3s localization to the nuclear surface is reduced in Msp300 RNAi clones marked with His–RFP. Quantification of Msp3s perinuclear signal: 132.8 ± 11.13 (n = 22 control) versus 30.5 ± 10.18 (n = 7 Msp300 RNAi clone), P = 0.000036. g, Shot localization to the perinuclear MTOC (arrow) is disrupted in Msp300 RNAi clones. h, Shot and Msp300 localization (antibody staining) to the perinuclear MTOC do not require Patronin. i, Msp300, Patronin and Msp3s (antibody staining) are delocalized to an ectopic MTOC in shot-knockdown cells. j, Double knockdown of Msp300 and shot with two combined fat body drivers (SPARC-Gal4 + Cg-Gal4) significantly diminished ectopic MT foci compared to shot knockdown alone. k, Quantification of ectopic MT foci in j and with two other combinations of RNAi knockdown. From left to right, P = 8.87 × 10−15, P = 0.0001, P = 1.62 × 10−15, n = 37, 33, 22 and 38 independent cells analysed, respectively. l, Msp300 and Patronin are delocalized to ectopic foci by shot RNAi; shot + Msp300 RNAi significantly reduced Msp300 and Patronin at foci. Msp300 signal: p = 3.44 × 10−3 (n = 8 shot RNAi; n = 18 double RNAi), Patronin signal: P = 1.69 × 10−15 (n = 9 shot RNAi; n = 20 double RNAi). m, Epistasis for key components. Experiments were performed three times in a, twice in g,h and five times in i. Quantification for b is shown in Fig. 3a. Images in c,d are representative of 30 and 14 independent clones, respectively. Data in e,f,k,l are presented as mean ± s.e.m; two-tailed Student's t-test; n = number of independent clones (e,f) or cells (k,l) analysed. Statistical details are presented in Source Data.
When fat body MTs were disrupted or if the MTOC was impaired, Collagen IV and other basement membrane proteins accumulated at higher levels at the plasma membrane of fat body cells and were visible in intact larvae (Fig. 6b) or dissected fat bodies (Fig. 6c–e and Extended Data Fig. 8a). Disruption of actin did not cause accumulation of basement membrane proteins (Extended Data Fig. 8b). As a consequence of fat body MT disruption, deposition of basement membrane components at the distant wing imaginal disc (Fig. 6f,g) were significantly reduced. Depletion of γ-TuRC components did not cause accumulation of basement membrane components on the plasma membrane (Fig. 6e), consistent with no involvement of the γ-TuRC in fat body MT organization.

The fat body ncMTOC is essential to restrict plasma membrane growth and avoid extracellular entrapment of basement membrane components. The accumulation of basement membrane proteins at the plasma membrane following disruption of the fat body ncMTOC occurs extracellularly, as demonstrated by antibody binding to Vkg–GFP on fixed but non-permeabilized fat body cells (Fig. 7a). Thus, basement membrane proteins are secreted but trapped on the outside of the plasma membrane. This phenotype of extracellular basement membrane entrapment occurs in fat body cells when endocytosis is disrupted45. When endocytosis is impeded, the plasma membrane overgrows (Fig. 7b,c) and becomes highly convoluted, thus trapping secreted collagen in the folds of the excess plasma membrane46. Disruption of the fat body MTOC caused similar overgrowth of the plasma membrane, as seen when endocytosis is blocked (Fig. 7b,c). Knockdown of Msps300 or msps, but not γTub23C, resulted in plasma membrane overgrowth (Fig. 7b) and entrapment of Collagen IV (Fig. 6e).

The fat body ncMTOC supports retrograde dynein-dependent endosome trafficking to maintain proper plasma membrane growth. Using GFP–Rab5 as an endosomal vesicle marker, we showed that in normal fat body cells, GFP–Rab5 vesicles were enriched at the plasma membrane and also encircled the nucleus where the ncMTOC resides (Fig. 7d). Following disruption of the fat body ncMTOC, the perinuclear pool of GFP–Rab5 vesicles was diminished and instead became elevated at the plasma membrane (Fig. 7d). Thus, fat body MT arrays are required for retrograde trafficking of endocytic vesicles from the plasma membrane to perinuclear sites.

Retrograde trafficking of endosomes requires the minus-end directed MT motor dynein in other systems46. Loss of retrograde dynein motor function, by dynein RNAi or overexpression of Dynamitin, shifted the perinuclear pool of GFP–Rab5 to the plasma membrane (Fig. 7d and Extended Data Fig. 8c). This blockade of endosomal trafficking by dynein inhibition phenocopied the effect of MT disruption on plasma membrane overgrowth (Fig. 7b,d) and entrapment of collagen at the plasma membrane (Extended Data Fig. 8d), but not nuclear mispositioning (Figs. 3a, 7d and Extended Data Fig. 8d), consistent with dynein being required for trafficking along MTs but not for the organization of the MT arrays. When the Kinesin-1 motor was knocked down to block anterograde MT trafficking, there was no effect on basement membrane secretion (Extended Data Fig. 8d) or nuclear positioning (Extended Data Fig. 8d). Therefore, a primary function of the fat body MTOC is to support the retrograde endosomal trafficking by dynein.

In summary, we show two key functions for the fat body MTOC: nuclear positioning and retrograde endosomal trafficking. These two functions are separable because blockade of endosomal machinery or dynein motor activity caused membrane thickening and basement membrane protein accumulation without affecting nuclear positioning.

Discussion

The rich diversity of non-centrosomal MT arrays and ncMTOC sites has long been recognized, but the molecular architecture of ncMTOCs and whether their composition and mechanisms of assembly are similar to those of the centrosome have been under-explored. Here we show that an ncMTOC assembles on the nuclear surface in Drosophila fat body cells (Extended Data Fig. 9a). A Nesprin–Shot complex anchors the ncMTOC at the nuclear surface to recruit the MT minus-end regulators Patronin and Nin for assembly, anchoring and/or stabilization of MT seeds that are elongated by Msps (Extended Data Fig. 9b). γ-Tubulin and known Msps cofactors, however, are notably not required. MT amplification by severing enzymes or the augmin complex are also not required for assembly. This work thus reveals unconventional paradigms for ncMTOC structure and for non-centrosomal MT assembly.

While it shares some properties with the muscle perinuclear MTOC47,48, such as stable MTs and the requirement for Nesprin and Shot, the fat body perinuclear ncMTOC is distinct in its molecular architecture and the mechanisms by which MT assembly is regulated. The fat body perinuclear MTOC differs from the muscle perinuclear MTOC in that the muscle requires Ensectin (known as MAP7 in humans)49, CLIP-19050, EB151, Kinesin-152 and Dynein53 for myonuclear positioning or morphology, but these proteins are not required for fat body MTOC organization or nuclear positioning (Supplementary Table 1). Functionally, the fat body ncMTOC maintains nuclear centricity and also serves a critical function by providing a trafficking conduit for dynein motor-based retrograde transport of endocytic vesicles, whose trafficking maintains the balance of plasma membrane growth (Extended Data Fig. 9c). Maintaining proper balance of membrane growth is an important function of the fat body MTOC to support the secretion of large molecules beyond the fat body. Large protein assemblies such as collagen IV and other basement membrane components become trapped in the membrane folds resulting from plasma membrane overgrowth when the MTOC is impaired.

Online content

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Fig. 6 | The fat body ncMTOC is essential for secretion of basement membrane proteins. a. Diagram of basement membrane component Collagen IV production in fat bodies and its secretion and deposition at basement tissues as a backbone membrane. b. Difference interference contrast (DIC) and fluorescent imaging of Collagen IV α2 (Vkg–GFP) in live fat bodies labelled with the membrane marker myr–RFP from control larvae and larvae with fat body-specific knockdown of Msps. Images are representatives of 12 larvae. c. Images showing fat body accumulation of indicated basement membrane components after MT disruption in fat body cells by αTub84B RNAi. GFP and yellow fluorescent protein (YFP) fluorescence indicate Vkg–GFP (Collagen IV α2) and Trol (Perlecana)–YFP, respectively. LanB1–sGFP and Ndg–sGFP are stained with anti-GFP antibody. d. Images showing fat body accumulation of Cg25c (Collagen IV α1) in αTub84B RNAi clones marked with GFP. e. Images showing fat body accumulation of Vkg–GFP after the indicated RNAi in fat body. f. Images showing reduced wing disc deposition of Vkg–GFP after MT disruptions in fat body cells by αTub84B RNAi or spastin overexpression. g. Quantification of Vkg–GFP fluorescence intensity in f. Data are means ± s.e.m. (n = 6 independent experiments (control) and n = 4 independent experiments (αTub84B RNAi and spastin overexpression)). Two-tailed Student’s t-test. αTub84B RNAi versus control, P = 0.0016; spastin overexpression versus control, P = 0.0001. Statistical details are shown in Source Data. The experiments in c–e were repeated three times with similar results. Genotype details are shown in Supplementary Table 3.
ARTICLES

Vkg–GFP DNA
Trol–YFP DNA
LanB1–sGFP DNA
Ndg–sGFP DNA

Control

DIC

Vkg–GFP (Collagen IV α2)

myr–RFP

Larval wing disc after MT disruption in the fat body

DNA Vkg–GFP

GFP clone

Staining

Brain

Collagen IV secretion

Collagen IV deposition

Fat body

Imaginal disc

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Fig. 7 | The fat body ncMTOC is essential for retrograde dynein-dependent endosome trafficking to restrict plasma membrane growth. a, Vkg–GFP accumulation at the plasma membrane is extracellular in αTub84B RNAi fat body cells, as shown by immunostaining with or without detergent permeabilization. Vkg–GFP autofluorescence is shown in green and GFP antibody staining is shown in red. Lamin C staining is included as a control for cell permeabilization. b, Overgrowth of plasma membrane (marked by myr–RFP) in fat body cells after disruption of MT directly (αTub84B RNAi or spastin overexpression) or by impairing the ncMTOC (msps or Msp300 RNAi), inactivation of dynein motor (Dhc64c RNAi) or endocytic machinery (Rab5(S43N), dominant-negative Rab5, a positive control). Insets show merged images with Vkg–GFP at the plasma membrane. c, Overgrowth of plasma membrane using the membrane dye CellMask Orange in GFP-marked fat body cells that express dominant-negative Rab5 or αTub84B RNAi. Slice 1 and slice 2 are separate image sections from the same confocal image z-stack. The areas in dashed boxes for control (white) and GFP clone (green) plasma membrane are shown in the panels on the right. d, Images showing endosome distribution (GFP–Rab5) in the indicated fat bodies. GFP–Rab5 is distributed at two main sites that are proximal to the plasma membrane and nuclear surface (control), but shifts to localizing predominantly near the plasma membrane after MT disruption (spastin overexpression or msp5 RNAi) or inactivation of dynein motor by Dhc64c RNAi. Middle: magnified views of cells from the white boxed region in the top panel, shown to highlight the membrane and perinuclear Rab5–GFP localizations. Bottom: magnified views of membrane GFP–Rab5 from the middle panel (red rectangle). Note nucleus positioning is impaired (yellow arrows) in spastin overexpression or msp5 RNAi cells, but normal (white arrows) in Dhc64c RNAi and wild type. Experiments in a–d were repeated twice with similar results. Genotype details are shown in Supplementary Table 3.
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References

1. Till, M. M. L. & Blake-Hedges, C. & Zheng, Y. & Buchwalter, R. A. & Megraw, T. L. Centrosomal and non-centrosomal microtubule-organizing centers (MTOCs) in Drosophila melanogaster. Cells 7, e121 (2018).
2. Sanchez, A. D. & Feldman, J. L. Microtubule-organizing centers: from the centrosome to non-centrosomal sites. Curr. Opin. Cell Biol. 44, 93–101 (2017).
3. Muroyama, A. & Lechler, T. Microtubule organization, dynamics and functions in differentiated cells. Development 144, 3012–3021 (2017).
4. Martin, M. & Akhmanova, A. Coming into focus: mechanisms of microtubule minus-end organization. Trends Cell Biol. 28, 574–588 (2018).
5. Farache, D. & Emorine, L. & Haren, L. & Merdes, A. Assembly and regulation of γ-tubulin complexes. Open Biol. 8, 170266 (2018).
6. Kollman, J. M. & Merdes, A. & Mourey, I. & Agard, D. A. Microtubule nucleation by γ-tubulin complexes. Nat. Rev. Mol. Cell. Biol. 12, 709–721 (2011).
7. Oakley, B. R., Paolillo, V. & Zheng, Y. γ-Tubulin complexes in microtubule nucleation and beyond. Mol. Biol. Cell 26, 2957–2962 (2015).
8. Chen, J. V., Buchwalter, R. A., Kao, L. R. & Megraw, T. L. A splice variant of centrosomin contributes mitochondria to microtubule-organizing centers. Curr. Biol. 27, 1928–1940.e1926 (2017).
9. Flor-Parra, I. & Iglesias-Romerro, A. B. & Chang, F. The XMAP215 ortholog Alp14 promotes microtubule nucleation in fission yeast. Curr. Biol. 28, 1681–1691 (2018).
10. Thawani, A. & Kadzir, R. S. & Petry, S. XMAP215 is a microtubule nucleation factor that functions synergistically with the γ-tubulin ring complex. Nat. Cell Biol. 20, 575–585 (2018).
11. Gunzelmann, J. et al. The microtubule polymerase Stu2 promotes oligomerization of the γ-TuSC for cytoplasmic microtubule nucleation. eLife 7, e39932 (2018).
12. Albert, J. et al. A structural model for microtubule minus-end recognition and protection by CAMSAP proteins. Nat. Struct. Mol. Biol. 24, 931–943 (2017).
13. Goodwin, S. S. & Vale, R. D. Patronin regulates the microtubule network by protecting microtubule minus ends. Cell 143, 263–274 (2010).
14. Hendershott, M. C. & Vale, R. D. Regulation of microtubule minus-end dynamics by CAMSAPs and Patronin. Proc. Natl Acad. Sci. USA 111, 5860–5865 (2014).
15. Schoenfelder, K. P. et al. Indispensable pre-mitotic endocytomes promote aneuploidy in the Drosophila rectum. Development 141, 3551–3560 (2014).
16. Arrée, E. L. & Soulages, J. L. Insect fat body: energy, metabolism, and lipid metabolism. Annu. Rev. Entomol. 55, 207–225 (2010).
17. Drosophila melanogaster. Interorgan communication pathways in physiology: focus on Drosophila. Annu. Rev. Genet. 50, 539–570 (2016).
18. Hoshizaki, D. K. et al. Embryonic fat-cell lineage in Drosophila melanogaster. Development 120, 2489–2499 (1994).
19. Clark, I. E., Jan, L. Y. & Jan, Y. N. Reciprocal localization of Nod and kinesin correlates with centrosome and spindle integrity. Curr. Biol. 19, 816–826 (2009).
20. Sanchez-Huetars, C. et al. Non-centrosomal nucleation mediated by augmin organizes microtubules in post-mitotic neurons and controls axonal microtubule polarity. Nat. Commun. 7, 12187 (2016).
21. Cunha-Ferreira, I. et al. The HAUS complex is a key regulator of non-centrosomal microtubule organization during neuronal development. Cell Rep. 24, 791–800 (2018).
22. Yau, K. W. et al. Microtubule minus-end binding protein CAMSAP2 controls axon specification and dendrite development. Neuron 82, 1058–1073 (2017).
23. Wang, S. et al. NOCA-1 functions with γ-tubulin and in parallel to Patronin to assemble non-centrosomal microtubule arrays in C. elegans. eLife 4, e08649 (2015).
24. Delgelghy, N. & Stilbourne, J. & Bornens, M. Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. J. Cell Biol. 118, 1565–1575 (2001).
25. Mogerensen, M. M. & Malik, A. & Piel, M. & Bouckson-Castaing, V. & Bornens, M. Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. J. Cell Biol. 113, 3013–3023 (2000).
26. Zheng, Y. et al. The Sec17 sleeve and centrosomal protein Ninein localizes asymmetrically to the oocyte, rectum, and sperm tail, and is not required for normal development, behavior, or DNA damage response in Drosophila. Mol. Biol. Cell 27, 1740–1752 (2016).
27. Goldspink, D. A. et al. Ninein is essential for apico-basal microtubule formation and CLIP-170 facilitates its redeployment to non-centrosomal microtubule organizing centres. Open Biol. 7, 160274 (2017).
28. kamnanda, M. et al. Loss of the Drosophila Ninein-related centrosomal protein Bug2D causes mitotic defects and impairs embryonic development. Biol. Open 5, 1040–1051 (2016).
29. Lecland, N. & Hsu, C. Y. & Chenin, C. & Merdes, A. & Bierkamp, C. Epidermal growth factor regulates CAMSAP–Katanin Complex. Dev. Biol. 2014, 295–309 (2014).
30. Rosen, J. N. et al. The Drosophila Ninein homologue Bug2D cooperates with Enconsin in myosin positioning. J. Cell Biol. 218, 524–540 (2019).
31. Mejat, A. & Misteli, T. LINC complexes in health and disease. Nucleus 1, 40–52 (2010).
32. Khanal, I. Elbedowy, A. Diaz de la Loza Mdel, C. Fletcher, G. C. & Thompson, B. J. Shot and Patronin polarise microtubules to direct membrane traffic and biogenesis of microvilli in epithelia. J. Cell Sci. 129, 2651–2659 (2016).
33. Pastor-Pareja, J. & Xu, T. Shaping cells and organs in Drosophila by opposing roles of fat body-secreted Collagen IV and perlecan. Dev. Cell 21, 207–215 (2011).
34. Dai, J., Ma, M., Feng, Z. & Pastor-Pareja, J. C. Inter-adipocyte adhesion and signaling by Collagen IV intercellular concentrations in Drosophila. Curr. Biol. 27, 2729–2740.e2724 (2017).
35. Zhang, Y. et al. Plasma membrane overgrowth causes fibrotic collagen accumulation and immune activation in Drosophila adipocytes. eLife 4, 78175 (2015).
36. Granger, E., Mcnee, G., Allan, V. & Woodman, P. The role of the cytoskeleton and molecular motors in endosomal dynamics. Semin. Cell Dev. Biol. 31, 20–29 (2014).
57. Fant, X., Srsen, V., Espigat-Georger, A. & Merdes, A. Nuclei of non-muscle cells bind centrosome proteins upon fusion with differentiating myoblasts. PLoS ONE 4, 0008303 (2009).

58. Srsen, V., Fant, X., Heald, R., Rabouille, C. & Merdes, A. Centrosome proteins form an insoluble perinuclear matrix during muscle cell differentiation. BMC Cell Biol. 10, 28 (2009).

59. Gimpel, P. et al. Nesprin-1α-dependent microtubule nucleation from the nuclear envelope via Akap450 is necessary for nuclear positioning in muscle cells. Curr. Biol. 27, 2999–3009 (2017).

60. Espigat-Georger, A., Dyachuk, V., Chemin, C., Emorine, L. & Merdes, A. Nuclear alignment in myotubes requires centrosome proteins recruited by nesprin-1. J. Cell Sci. 129, 4227–4237 (2016).

61. Bugnard, E., Zaal, K. J. M. & Ralston, E. Reorganization of microtubule nucleation during muscle differentiation. Cell Motil. Cytoskel. 60, 1–13 (2005).

62. Elhanany-Tamir, H. et al. Organelle positioning in muscles requires cooperation between two KASH proteins and microtubules. J. Cell Biol. 198, 833–846 (2012).

63. Mao, C. X., Wen, X., Jin, S. & Zhang, Y. Q. Increased acetylation of microtubules rescues human tau-induced microtubule defects and neuromuscular junction abnormalities in Drosophila. Dis. Model. Mech. 10, 1245–1252 (2017).

64. Wang, S., Reuveny, A. & Volk, T. Nesprin provides elastic properties to muscle nuclei by cooperating with spectraplakin and EB1. J. Cell Biol. 209, 529–538 (2015).

65. Metzger, T. et al. MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. Nature 484, 120–124 (2012).

66. Folker, E. S., Schulman, V. K. & Baylies, M. K. Muscle length and myonuclear position are independently regulated by distinct Dynein pathways. Development 139, 3827–3837 (2012).

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Methods

Drosophila stocks. Information for the source, identifier, and original reference for all fly strains used, including mutants, RNAi lines, transgenic lines and drivers are listed in Supplementary Table 2. Detailed experimental genotypes and their associations with each figure are provided in Supplementary Table 3. Flies were maintained on standard food and incubated at 25 °C. RNAi-mediated knockdowns were crossed at 29 °C.

Plasmid constructs and generation of UASp-CFP-Mps- Drosophila strain. The coding sequences for Mps were amplified by PCR from cDNA clone LPL4448 (accession no. BT023496), cloned into pENTR-D’Topo (Thermo Fisher), and sequenced before shuffling into pPCW-attB (a gift from M. Buszczak, UT Southwestern Medical Center) to generate UASp-CFP-Mps, and into pPHW-attB to generate UASp-HA-Mps. UASp-CFP-Mps plasmid was injected into embryos for targeted insertion on chromosome 3 at ZH-96E by Bestgene.

Generation of mutant and FLP-out expression clones in fat bodies. Gain-of-function (Fli-out) clones were generated by crossing virgin females: hsFlp; UAS-Δ2; Act > CD2 > Gal4, UAS-GFP / TM6B or hsFlp; Act > CD2 > Gal4, UAS-His-RFP/TM6B to UAS-driven RNAi lines or other transgenes. FLP-mediated excision of the CD2 insert induces gene knockdown or overexpression driven by Act-Gal4 with UAS-Δ2 at 29°C in GFP-marked cells. These clones were generated in early embryos by a shift to 29°C, and fat body precursor cells as evidenced by clones that typically include multiple cells due to mitotic expansion of the clone prior to differentiation.

hsFLP/Flippase recognition target (FRT)-mediated Msg300 loss-of-function clones in larval fat body were induced in 0–6 h embryos by a 1 h heat shock at 37°C using an FRT-linked Msg300mutant allele. Mutant clones were marked by loss of GFP.

Antibodies. All antibodies used are described in Supplementary Table 5.

Immunostaining. Most staining was performed in fat bodies from late third-instar wandering larvae; early stages of larvae were included in Extended Data Fig. 4d–e. Staining of MTOC components in larval fat bodies was performed after methanol fixation as previously described, with minor modification35. Fat bodies were dissected from late third-instar wandering larvae in 1× phosphate-buffered saline (PBS). The dissected fat bodies from one larva were transferred to 8 µl of 100 mM PIPES, pH 6.9, 1 mM EGTA, and 2 mM MgSO4 on a slide. A 22 × 22 mm siliconized coverslip (NA) oil-immersion objective. All confocal images were captured with a spacing of 0.25 µm per z-stack.

Transmission electron microscopy. Transmission electron microscopy analysis of the MTS at the fat body nMTOC was performed essentially as described17, except that fat bodies from 3rd–3rd instar larvae were fixed for 72 h in 1 M Karmovsky’s fixative (EM Sciences, cat. no. 15720). Embedding, staining, sectioning and preparation of grids were performed by the Core Facility at University of Texas Southwestern Medical Center. After three rinses with 0.1 M sodium cacodylate buffer, samples were embedded in 3% agarose and sliced into small blocks (1 mm3), rinsed with the same buffer three times and post-fixed with 1% osmium tetroxide and 0.8% uranyl acetate in ferricyanide for 1.5 h at room temperature. Samples were rinsed with water and en bloc stained with 4% uranyl acetate in 50% ethanol for 2 h. They were then dehydrated with increasing concentration of ethanol, transitioned into propylene oxide, infiltrated with Embed-812 resin and polymerized in a 60 °C oven overnight. Blocks were sectioned with a diamond knife (Diatome) on a Leica Ultracut U ultramicrotome (Leica Microsystems) and collected onto copper grids, post stained with 2% aqueous Uranyl acetate and lead citrate. Images were acquired on a Phillips CM12 BioTwin transmission electron microscope at the Florida State University Biological Science Imaging Resource (BSIR).

Co-immunoprecipitation. Co-immunoprecipitation of HA–Mps and Patronin was performed with Drosophila S2 cells. S2 cells were cultured in Shilds and Sangs M3 medium (Sigma, cat. no. S3652) with 10% fetal bovine serum (Gibco, cat. no. 10437-010) at room temperature. pMT-GAL4 (DGRC, cat. no. 1042) used to induce expression from pUASp-HA-Mps. Plasmids (pMT-GAL4 plus or minus pUASp-HA-Mps) were transfected using Lipofectamine 3000 (Thermo Fisher) in six-well dishes. After 48h of culture, expression was induced by addition of 1 mM CuSO4, for 24 h. Cells were collected and lysed in 500 µl lysis buffer: 50 mM Tris pH 8, 150 mM NaCl, 1 mM DTT, 0.5% NP-40 plus protease inhibitor cocktail (Sigma, cat. no. P8340) for 1 h on ice. Immunoprecipitation was performed using anti-HA–agarose (Sigma, cat. no. 2098) following the manufacturer’s recommendations. For western blots, input lanes represent 2.1% of the total lysates and immunoprecipitation lanes represent 62.5% of the total immunoprecipitate. Mouse anti-HA (HA-7, Sigma, cat. no. 19658, 1:40,000) and rabbit anti-Patronin14 (1:500) were used to detect HA–Mps and endogenous Patronin, respectively. Western blotting was performed as described below.

Western blot. Fat bodies or brains dissected from five wandering larvae were lysed in 50 µl (fat bodies) or 10 µl (brains) of 2× SDS–PAGE buffer (100 mM Tris-HCL, pH 6.8, 4% SDS, 0.02% bromphenol blue, 20% glycerol and 5% β-mercaptoethanol). Ten whole larvae at wandering stage were lysed with a motorized pestle in 200 µl of 2× SDS–PAGE loading buffer. After boiling for 5 min, larval lysates were centrifuged at 13,200g for 5 min to clear pellets. Ten microliters of larval lysates were loaded for SDS–PAGE, followed by semi-dry transfer to nitrocellulose membrane. The membranes were blocked with 5% non-fat milk in 1X TBS for 1 h at room temperature and then probed with primary antibodies diluted in 1X TBS containing 0.1% Tween (v/v). Blots were scanned on an Odyssey Infrared Imaging system (LI-COR Bioscience). Signals were quantified using Li-Cor Image Studio software. Images were processed in Adobe Photoshop CS4 and presented in monochrome.

Microtubule regrowth assay. Wild-type fat bodies were dissected and placed in 15 µl of 10 mg ml−1 fibrinogen (EMD, cat. no. 341573) in Dulbecco’s PBS (D-PBS, Invitrogen) on a clean slide with a hydrophobic ring drawn with a super PAP pen. Two microliters of thrombin solution (Sigma T9549–50UN; 100 U ml−1) in D-PBS was pipetted to the fibrinogen solution to induce a fibrin clot. The fat bodies were treated with 30 µl vinblastine in Shilds and Sangs M3 medium (Sigma, cat. no. S3652) for 1–2 h to induce MT disassembly at room temperature. The fibrin clot was washed out in ice-cold D-PBS (0 °C, or at a constant 4 °C) for 1 h, followed by a time-course recovery at 25 °C. The fat bodies were fixed at the respective time points in →0°C methanol for 10 min and stained with anti-α-Tubulin and DAPI to label MTs and nuclei, respectively. Microtubule regrowth in the γ Tub23C RNAi clone experiment (Extended Data Fig. 4f) was fixed with 4% PFA to enable staining with CF568–phalloidin.

Image acquisition. Fixed fat body samples were imaged using a Nikon A1 laser scanning confocal microscope (Nikon, Japan) using a x60 1.49 numerical aperture (NA) oil-immersion objective. All confocal images were captured with a spacing of 0.25 µm or 0.5 µm between z-sections using Nikon NIS-Elements AR software (v.4.6) and are presented as maximum intensity projections of z-stacks. Images of larval nuclei and brains were acquired by stitching together images acquired with 25% overlap using in Elements using a x60 1.49 NA oil-immersion objective. Gamma-correction was applied to images.

Live imaging of intact fat body in whole larval expressing vgk–GFP and myr–RFP was performed with a Macro Zoom microscope (MVX10, Olympus) equipped with a DP72 camera. The DIC view of whole larva was imaged at 100X and magnification. Fluorescent imaging of Vg-actin expressing in larval fat bodies at single-cell resolution was performed at 130-fold magnification. Representative still frames are shown in Fig. 6b.

Live imaging of nuclear positioning or movement in intact fat body expressing myr–RFP and Histone-GFP was performed on whole live third-instar wandering stage larva reared between a 22 × 22 mm and a 24 x 40 mm 1.5 coverslip using clear 0.25 inch wide office tape. Images were captured every 3.0 s using 488 nm and 568 nm laser excitation with a x20 0.75 NA Plan Apo objective on a Nikon A1 confocal microscope with the pinhole open to 8.0. The time-lapse image sequence was converted to .avi video at 20 frames per s.
Quantification analysis. Nuclear positioning or centricity analysis in fat bodies was measured as the distance between the geometric centres of the cell and the nucleus using Nikon NIS-Elements AR software (v.4.6). Cell and nuclear geometric centres were measured using the ‘centroid’ tool after automatic or manual thresholding of the cell boundary (cortical F-actin by Phalloidin staining) and nucleus (Hoechst staining). The distance between cell and nuclear geometric centres was measured using the length tool in NIS-Elements.

Fluorescent intensity of circumferential and radial MTs, Msps or Patronin in GFP or His–RFP-marked clones was analysed in ImageJ. Integrated density and area for circumferential or radial MTs was measured in 8-bit inverted monochrome images. Mean fluorescence intensity was measured as integrated density per unit area using the measure tool in ImageJ on 8-bit monochrome inverted and thresholded images. For each RNAi knockdown, analysis was performed in control and GFP-marked clones for side-by-side comparison.

Vkg–GFP fluorescent intensity in larval wing discs was quantified in ImageJ. Mean fluorescent intensity of GFP autofluorescence was measured after GFP signal thresholding in 8-bit inverted images.

Statistics and reproducibility. Sample size was chosen according to standard practices in the field to ensure adequate statistical power. No data were excluded from our studies. For experiments where image parameters were quantified, a minimum of four independent images were quantified and in most experiments it was eight or more. Almost all of the images were examined as clones: experimentally manipulated cells generated within an otherwise wild-type tissue. This approach adds notable and significant rigor to the experimental design and the measurements taken, as the control cells are measured side-by-side with the experimental cells. The numbers (n) measured were significant as indicated by the low P values (<0.01) generated from the data. Data are presented as mean ± s.e.m. The number of replicates and sample sizes are indicated in the figure legends. Statistical significance was determined by unpaired two-tailed Student’s t-test using Prism 7 (Graphpad). Differences were considered statistically significant when P < 0.05. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 as shown in figures.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. Data not included are available from the corresponding authors upon reasonable request.

References
67. Chen, J. V. et al. Rootletin organizes the ciliary rootlet to achieve neuron sensory function in Drosophila. J. Cell Biol. 211, 435–453 (2015).

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Author contributions
T.L.M. and Y.Z. designed the study. Y.Z. performed and analysed most experiments. R.A.B. and J.V.C. performed and analysed centrosomal protein staining. C.Z. performed and analysed coimmunoprecipitation assays. E.M.W. imaged and analysed transmission electron microscopy data. Y.Z. and T.L.M wrote and revised the manuscript with constructive input from all authors.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | Fat body MTs are stabilized. (a) Additional EM images, related to Fig. 1c. (b) Images of IF stained fat body cells with or without cold treatment for 1 hr, which did not cause significant reduction of the MT array. (c, d) IF staining shows that the fat body contains MTs that are acetylated (c) and polyglutamylated (d), two post-translational modifications attributed to stabilized MTs. Genotype details are in Supplementary Table 3. Experiments were performed once in (a), twice in (b–d) with similar results. Scale bar, 1 μm (a), 20 μm (b–d).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Survey of localization of centrosomal proteins and MT regulators at the fat body ncMTOC. Images of fat body cells stained for Cnn and counterstained in green for the indicated proteins using either antibody staining or expression from a fluorescent protein-tagged transgene as indicated. The insets are Cnn staining as positive control. See Supplementary Table 1 for the complete list of proteins assayed and the summary of their localization at the MTOC as well as the information about promoters used to drive transgenes. Anti-GFP or anti-RFP staining was applied for GFP-tagged or RFP-tagged transgenes. The centriolar proteins are core centriolar proteins. The “PCM + centriolar” proteins are proteins that reside in both compartments or straddle both compartments and are known to function in PCM organization. The “MT regulatory” are effector proteins with established roles in regulating MT assembly or anchoring. Since some of the transgenic proteins were expressed ectopically, it is possible that some of the positive localizations determined in this manner do not reflect endogenous protein localization. Collectively, the fat body MTOC contains some proteins in common with the centrosome PCM, but also some distinct components including Patronin. Note that Ana1-GFP image in Fig. 3b is reproduced from this figure. Genotype details are in Supplementary Table 3. Staining experiments were performed twice with similar results. Scale bar, 20 µm.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Single or double mutant combinations of centrosomal protein genes do not overtly impair MT assembly at the fat body ncMTOC. Fat body cells were stained for DNA (Hoechst) and filamentous actin (CF568-Phalloidin) to assay for nuclear centricity (top panels). MTs (anti-α-tubulin) and DNA (DAPI) were stained to assay MT organization with respect to nuclei (bottom panels). None of the single or double mutants tested shows defects in nuclear positioning or MT assembly at the MTOC. Nuclear positioning (top panel) are quantified in Fig. 3a. Genotype details are in Supplementary Table 3. Experiments were performed twice with similar results. Scale bar, 50 µm (top panels), 20 µm (bottom panels).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | γ-tubulin is not required for MT assembly at the fat body ncMTOC. (a) Western blot detection of γ-tubulin in wild-type and γTub23C mutant larval fat body and brain lysates. Wild type is w1118, γTub mutant is γ-Tub23C^{GC2} /Df(2L)JS17. Triose-phosphate isomerase (TPI) is the loading control. γ-tubulin levels, as measured from the shown blot, were about 120-fold lower in wild-type larval fat bodies compared to brains when normalized against Tpi. (b) Western blot detection of γ-tubulin in whole larval lysates from wild-type and three independent γTub23C RNAi lines driven by two ubiquitous strong promoters: Tub-Gal4 and with Act5c-Gal4 plus UAS-Dicer-2. (c) Images showing γ-tubulin staining in γ-Tub23C^{GC2} RNAi fat body clone marked by His-RFP. (d, d’) Images showing γ-tubulin staining in wild-type (d) and γ-Tub23C mutant (d’) fat body at different developmental stages as indicated. (e, e’) Images showing nuclear positioning and MT assembly in wild-type (e) and γ-Tub23C mutant (e’) fat body cells. (f) Images showing MT regrowth in wild-type and γ-Tub23C depleted fat body cells from γ-Tub23C^{GC2} RNAi clones marked by His-RFP. Control: no vinblastine treatment; the other three groups were treated with vinblastine and underwent MT regrowth for 0, 5, 30 min as indicated. The western blots in a and b, staining in c, d, d’, e, e’, f were repeated twice with similar results. Full blots for a, b are shown in Source Data Extended Data Fig. 4_Uncropped Western Blots. Scale bar, 50 μm (left panels in c, top panels in d, e’, top panels in f), 10 μm (right panels in c, bottom panels in d, e’), 20 μm (bottom panels in f).
Extended Data Fig. 5 | Msps localization at the fat body ncMTOC does not require TACC, AurA or Ncd. (a) Staining for Msps in msps RNAi fat body clones marked by His-RFP. Arrow indicates the loss of Msps in an msps RNAi cell. (b) Msps localization at centrosomes or spindle poles relies on TACC, Aurora A kinase phosphorylation of TACC, and the Kinesin-14 motor Ncd. None of these components are required for Msps localization at the fat body ncMTOC (top and middle panels), or for fat body nuclear positioning (bottom panels). Fat bodies were stained with antibodies against Msps and Cnn, or stained for DNA (DAPI), MTs using a FITC-conjugated DM1A antibody, and actin with CF568-conjugated phalloidin. Staining was repeated twice in a, three times in b with similar results. Nuclear positioning in b was quantified in Fig. 3a. Genotype details provided in Supplementary Table 3. Scale bar, 50 μm (a, bottom panels in b), 10 μm (top panels in b).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Patronin cooperates with Nin but not with γ-tubulin or other known Patronin interactors in fat body. (a) Western blot detection of Patronin in larval fat body lysates from wild-type and Patronin knockdown. Arrows indicate the established Patronin isoform at 180 kDa, and another putative isoform at approximately 65 kDa. (b) MT staining in wild-type, single knockdown of Patronin or Klpl0A (Kinesin-13), and double knockdown of Patronin plus Klpl0A. (c) Mutants for MT severing enzymes katanin p60 or katanin p60-like 1 do not affect nuclear positioning (top) or MT assembly (bottom). (d) Knockdown of MT severing enzymes kat80 (c) or spastin (d) or double knockdown of kat80 plus spastin does not affect nuclear positioning (top) or MT assembly (bottom). (e) Mutant for dgt4 does not affect nuclear positioning (left) or MT assembly (right). (f) γTub23C mutation together with Patronin RNAi does not significantly affect nuclear positioning (left) or radial MTs (right). (g) Western blot detection of Nin in larval brain lysates from wild-type, Nin RNAi, and Nin mutant. (h) Nin staining in Nin RNAi fat body clone marked by His-RFP. (i) Nin, Patronin and MTs in γTub23C mutant fat body. (j) Double knockdown of Nin and γTub23C, or Nin and gcp3 does not affect nuclear positioning and MT assembly in fat body clones. The western blots in a and g were performed twice with similar results. Full blots are shown in Source Data Extended Data Fig. 6_Uncropped Western Blots. The staining were repeated three times in (b, c, e, i), twice in (d, f, h, j) with similar results. Scale bar, 20 µm (b, bottom panel in c, middle and bottom panels in d, right panel in h, i, top panels in j), 50 µm (top panels in c-e, left panels in f, h), 10 µm (magnified panels in j).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Shot depletion generates an ectopic MTOC and results in severe and pleiotropic disruption of subcellular compartments. (a) Shot knockdown results in a reorganization of the MTOC from the nuclear surface to a centrosome-like focus in the cytoplasm (arrows). (b, c, d) Cnn (b), γTub23C (c) and Klar (d) are not delocalized from the nuclear surface to the ectopic MTOC (arrows) generated by shot knockdown. Arrow in c indicates no γTub23C-GFP enrichment in the ectopic centrosome-like MTOC. (e) Actin ‘collapses’ into an aggregate (arrows) near the MTOC after shot knockdown. Note that nuclear centricity is also affected. Inset shows the merged image with MTs. (f, g) Nuclear morphology (f) and organization of organelles (g) are disrupted in shot knockdown fat bodies. Arrows point to accumulation of ER (RFP-KDEL), Golgi (ManII-GFP) and mitochondria (anti-ATP5α) near actin aggregates. (h) Shot knockdown results in accumulation of basement membrane components in the cytoplasm, proximal to the ectopic MTOC (arrows). (i) Deposition of collagen IV α2 (Vkg-GFP, arrows) on the wing disc is decreased when shot is knocked down in fat body cells. (i') Quantitation of Vkg-GFP in (i). Data are the means ± s.e.m (n = 6 independent experiments), p = 0.000088 (****) by two-tailed Student’s t-test. Statistical details are shown in Source Data Extended Data Fig. 7_Statistical Source Data. Experiments in a–h were performed three times with similar results. Genotype details are in Supplementary Table 3. Scale bar, 50 μm (a, e–i), 20 μm (b–d).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Supporting data for secretion of basement membrane components and retrograde trafficking as well as plasma membrane growth. (a) Images showing fat body accumulation of different basement membrane components after disruption of MTs in fat body cells with β-Tub56D RNAi or spastin overexpression. (b) Images showing no accumulation of different basement membrane components after actin disruption in fat body by Arp2 RNAi. (c) Images showing that GFP-Rab5 endosomes shift from their perinuclear location (yellow arrowheads) to the plasma membrane site (white arrows) after inactivation of dynein activity (Dynamitin overexpression). Conversely, dynamin loss (shi RNAi) disrupts endocytic vesicle budding, causing a loss of GFP-Rab5 vesicles at the plasma membrane. (d) Images showing fat body accumulation of Vkg-GFP at the plasma membrane after inactivation of dynein activity either by Dhc64c RNAi or Dynamitin overexpression, but not after inactivation of kinesin-1 activity by Kinesin heavy chain (Khc) or Kinesin light chain (Klc) RNAi. Experiments in a–d were repeated twice with similar results. Genotype details are in Supplementary Table 3. Scale bar, 50 µm.
Fat body ncMTOC assembly and function

Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Summary and model for perinuclear non-centrosomal MTOC assembly and function in fat body cells. (a) *Drosophila* fat body cells, a differentiated cell type analogous to human adipocytes and liver, assemble a perinuclear ncMTOC that organizes dense circumferential MTs and radial MTs. The radial MTs are polarized, emanating from the nuclear surface (minus-end) towards the plasma membrane (plus end). N: nucleus. (b) The perinuclear ncMTOC has unique architecture and MT assembly mechanisms. Msp300/Nesprin anchors the ncMTOC at nuclear surface, requiring and recruiting Shot, and is epistatic to Patronin/CAMSAP. Patronin and Nin cooperate to recruit Msps for radial MT elongation independently of γ-tubulin. Domains are shown for each protein. (c) The perinuclear ncMTOC has two critical physiological functions: nuclear positioning and control of plasma membrane growth via retrograde trafficking. The fat body perinuclear ncMTOC controls endosomal retrograde trafficking in coordination with minus-end directed dynein to restrict plasma membrane overgrowth. Disruption of the ncMTOC or inactivation of dynein blocks retrograde membrane trafficking, leading to excessive plasma membrane growth and convoluted “thickened” plasma membranes. Consequentially, secreted BM components are trapped within the convoluted plasma membrane folds. The entrapment of BM proteins in fat body cells leads to reduced BM deposition in destination tissues, including imaginal discs and brains (not depicted).
Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: Nikon NIS Elements software AR 4.6; Li-Cor Image Studio (version 5.2.5)

Data analysis: GraphPad Prism (version 7.0); Image J (version 1.51j8); Adobe Illustrator CC 2015; Adobe photoshop CS3; Nikon NIS Elements software AR 4.6; Li-Cor Image Studio (version 5.2.5)

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: Sample size was chosen according to standard practices in the field. For experiments where image parameters were quantified, a minimum of 4 independent images were quantified and in most experiments it was 8 or more. Almost all of the images were examined as clones. Experimentally manipulated cells were generated within an otherwise wild-type tissue. This approach adds notable and significant rigor to the experimental design and the measurements taken, as the control cells are measured side-by-side with the experimental cells. The numbers (n) measured were significant as indicated by the low p values (<0.01) generated from the data.

- **Data exclusions**: No data were excluded from our studies.

- **Replication**: All attempts at replication were successful. Experiments were carried out at least three times unless otherwise indicated.

- **Randomization**: Not necessary or applicable for our approaches to achieve rigorous experimental results. Much of our analysis was performed in mosaic clones, permitting well-controlled wild type vs mutant/condition comparisons.

- **Blinding**: Also not necessary or applicable for our approaches to achieve rigorous experimental results. Much of our analysis was performed in mosaic clones, permitting well-controlled wild type vs mutant/condition comparisons. Multiple controls were employed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | Involved in the study |
| - Antibodies | | - Involved in the study |
| | - Eukaryotic cell lines | | - ChiP-seq |
| | - Palaeontology | | - Flow cytometry |
| | - Animals and other organisms | | - MRI-based neuroimaging |
| | - Human research participants | |
| | - Clinical data | |

Antibodies used

- Mouse anti-α-tubulin (DM1A; MilliporeSigma, St. Louis, MO, Cat#T9026, Lot #057K4842)
- Rat monoclonal anti-α-tubulin (Y1/2; Thermo Fisher, Waltham, MA, Cat#MA1–B007, Lot#T2545532)
- FITC-conjugated α-tubulin (DM1A; MilliporeSigma, Cat#F2168, Lot#)
- Mouse anti-β-tubulin (E7; Developmental Studies Hybridoma Bank [DSHB], University of Iowa; Lot#1/12/17)
- Mouse anti-acetylated tubulin (6-11B-1; MilliporeSigma; Cat#6793, Lot#056K4771)
- Mouse monoclonal anti-glutamylated tubulin (b3; MilliporeSigma; Cat#T9822, Lot#2K4842)
- Mouse anti-tubulin (STU88; MilliporeSigma; Cat#76557, Lot#2K4842)
- Rabbit or guinea pig anti-α-Chn (Kao and Megraw, 2009)
- Mouse monoclonal anti-lamin C (L228.28; DSBH, Lot#4/2/16)
- Mouse monoclonal anti-β Gal (40 1a; DSBH, Lot#4/19/18)
- Mouse monoclonal anti-GFP (366; Cat#A-11120, Invitrogen, Lot#567264)
- Chicken anti-GFP (Cat#GFP-1020, Aves labs, Lot#0316F111)
- Rabbit anti-RFP (Cat#AB32216, Chemicon, Lot#06047035538)
- Rabbit anti-TPI (Cat#Wic-30145, Santa Cruz Biotechnology; discontinued)
- Rabbit anti-Msp80 (Kao and Megraw, 2009)
- Rabbit anti-Msp80 (Currie et al., 2011)
- Guinea pig anti-Msp300 (Elhanany-Tamir et al., 2012)
- Mouse anti-ATP6alpha (1SH4C4; Cat#ab14748, Abcam; Lot#)
- Mouse anti-Ki67 (9C10; DSBH, Lot#1/4/15)
- Mouse anti-Shh (mAbR01); DSBH; Lot#0/24/16)
guinea pig anti-Ninein (Jampietro et al., 2014)
rabbit anti-Patronin (Goodwin and Vale, 2010)
rabbit anti-Skp2 (Dix and Raff, 2007)
rabbit anti-Grip84/GCP2 (Ogema et al., 1999)
rabbit anti-Grip91/GCP3 (Ogema et al., 1999)
rabbit anti-Grip128/GCP5 (Ogema et al., 1999)
rabbit anti-centrocortin (Kao and Megraw, 2009)
guinea pig anti-Cg25C (Shahab et al., 2015)
rabbit anti-ensconsin (Barlan et al., 2013)

Secondary Antibodies:
Alexa Fluor 488 Goat Anti-Mouse IgG (Cat#A11029, ThermoFisher; lot #1423008)
Alexa Fluor 568 Goat Anti-Mouse IgG (Cat#A11031, ThermoFisher; lot #1398018)
Alexa Fluor 647 Goat Anti-Mouse IgG (Cat#A21236, ThermoFisher; lot #1793803)
Alexa Fluor 488 Goat Anti-Rabbit IgG (Cat#A11034, ThermoFisher; lot #1880241)
Alexa Fluor 568 Goat Anti-Rabbit IgG (Cat#A11036, ThermoFisher; lot #1924788)
Alexa Fluor 647 Goat Anti-Rabbit IgG (Cat#A212245, ThermoFisher; lot #1700082)
Alexa Fluor 488 Goat Anti-Guinea Pig IgG (Cat#A11073, ThermoFisher; lot #38320A)
Alexa Fluor 568 Goat Anti-Guinea Pig IgG (Cat#A11075, ThermoFisher; lot #722782)
Alexa Fluor 647 Goat Anti-Guinea Pig IgG (Cat#A212450, ThermoFisher; lot #201614D)
Alexa Fluor 488 Goat Anti-Rat IgG (Cat#A11006, ThermoFisher; lot #198714B)
Alexa Fluor 568 Goat Anti-Rat IgG (Cat#A11077, ThermoFisher; lot #870956)
Alexa Fluor 647 Goat Anti-Rat IgG (Cat#A21247, ThermoFisher; lot #1611119)
Alexa Fluor 488 Goat Anti-Chicken IgG (Cat#A11030, ThermoFisher; lot #41658A)

Validation
Antibodies were validated by indirect immunofluorescence staining or western blotting of mutant or RNAi knockdown specimens compared to normal or wild type controls. In the case of tags derived from other species, expression of the tagged molecule was compared with specimens where the tagged molecule was not expressed.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals
This study used Drosophila melanogaster, which do not require Animal Care and Use Committee (ACUC) oversight. Many different mutant and transgenic strains were used, and all are listed in Supplementary Table 1. All animals used in the study were at the third instar larval developmental stage, with the exception of the examination of first and second instar larvae in experiments shown in Extended Data Figure 4. Mixed sex animals were used, and data were not analyzed differentially with respect to sex.

Wild animals
This study did not involve wild animals.

Field-collected samples
This study did not involve samples collected from field.

Ethics oversight
No ethical approval is required for research using Drosophila as a model.

Note that full information on the approval of the study protocol must also be provided in the manuscript.