Katanin p60-like 1 Sculpts the Cytoskeleton in Mechanosensory Cilia

Landi Sun, Lihong Cui, Zhen Liu, Qixuan Wang, Zhaoyu Xue, Menghua Wu, Tianhui Sun, Decai Mao, Jianquan Ni, Jose Pastor-Pareja, and Xin Liang

Corresponding Author(s): Xin Liang, Tsinghua University

Review Timeline:

| Event                  | Date          |
|------------------------|---------------|
| Submission Date        | 2020-04-21    |
| Editorial Decision     | 2020-07-06    |
| Revision Received      | 2020-09-28    |
| Editorial Decision     | 2020-10-13    |
| Revision Received      | 2020-10-16    |

Monitoring Editor: Mark Peifer

Scientific Editor: Melina Casadio

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.202004184
Dear Prof. Liang,

Thank you for submitting your manuscript entitled "Dual Functions of Katanin p60-L1 in Sculpting the Cytoskeleton in Fly External Mechnosensory Cilia". We are truly very sorry for the significant and unusual delay in processing the paper and sending our decision to you. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers’ key concerns, as outlined here.

As you will see, all of the reviewers found the work to be potentially of significant interest and the data of high quality. All had suggestions, most of which can be addressed by changes to the text. Reviewers #1 and #2 had some experimental suggestions for strengthening the mechanistic underpinnings of the behavioral phenotypes -- finding ways to address these would strengthen the work.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

***IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.***

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations
are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to the Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,
Mark Peifer, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

Living organisms need mechanosensation for survival and force perception. The role of cytoskeleton in mechanosensation is barely studied and remains elusive. In this manuscript, the authors reconstructed the near-molecular resolution structure of the microtubule based cytoskeleton in the sensory cilium of fly campaniform mechanoreceptors using electron tomography, and resolved two ordered microtubule arrays in the outer segment. To investigate how the microtubule structure is formed, the authors found the microtubule severing enzyme Kat-60L playing a dual role sculpting the microtubule structure in campaniform mechanoreceptors, which in turn regulates the mechanosensitivity of these receptors. This is an interesting study and there are a lot of nice data presented in this paper. I think it is suitable for JCB provided the authors can address a few issues.

Specific questions:
1. Katanins including Kat-60L1 have been shown to play an important role in neuron development such as axonal growth and dendrite pruning. How do the authors know the reduced mechanosensitivity of the haltere receptors in the kat-60L1 mutant is not due to general developmental/morphological defect of the receptors? In fact, kat-60L1BE6/PBac mutant larvae are also less sensitive to noxious heat (Reference 15). And here Fig 4E-F does show that the MO in the kat-60L1 mutant is somewhat structurally deformed. What is the mechanosensitivity of the mutant
leg receptors that maintain a wild type-like structure?
2. Kat-60L1 expression is not only found in mechanosensory neurons but also in CNS. How do the authors know the flight behavior defect results from the defect in sensory neurons? More specific driver other than the pan-neuronal driver elav-Gal4 is needed in the rescue experiment.
3. The authors used FRAP assay to show that microtubule is stable in the outer segment in Fig 2F. Previous study from the Parrish lab showed a positive correlation between microtubule stability and Drosophila larval mechanosensitivity (Yan et al., 2018). As the authors think Kat-60L1 plays a role in regulating mechanosensitivity as well, is microtubule stability affected in the c01236/BE6 mutant?
4. If the outer segments of bristle and campaniform mechanoreceptors share a similar organization, why is NompC positive region reduced in the outer segment of campaniform mechanoreceptor while much expanded/elongated in that of bristle mechanoreceptor?
5. Is Kat-60L1 expressed in S2 cells? Could Kat-60L1 affect mechanosensitivity of S2 cells with ectopic expression of mechanosensitive channels like NompC? If there is no endogenous Kat-60L1, what happens if co-expressing NompC and Kat-60L1?

Minor questions:
1. A standard abbreviation of katanin p60-like 1 in flybase and other literatures is kat-60L1 instead of kat p60-L1. The authors should follow flybase.
2. Figure3F should have a negative control that is supposed to have no response (eg. nompC mutant).

Reviewer #2 (Comments to the Authors (Required)):

Review of Dual Functions of Katanin p60-L1 in Sculpting the Cytoskeleton in Fly External Mechanosensory Cilia by Sun et al.

In this manuscript, the authors' goal was to determine how the microtubule cytoskeleton contributes to mechanosensation using fly halter and leg campaniform mechanoreceptors as models. They began their study with an impressive set of experiments using use high-resolution tomographic EM to describe microtubule organization in the outer segments of these cells and identified two independent microtubule arrays that appear to be common to both cell types. Microtubules in the outer segments appear to be extensively cross-linked with each other and with a basket of peripheral doublet microtubules by poorly-resolved electron-dense structures. They then used the minus end binding protein Patronin to establish that microtubules in the outer segments exhibit a uniform polarity with their plus ends distal to the cell bodies and this observation was further supported by imaging of EB1, a plus end marker. These analyses added further evidence to the existence of two disparate microtubule networks as the EB1 trajectories in the inner segments revealed microtubules in this compartment are oriented with their minus ends distal to the centers of the cells. The authors next examined microtubule turn-over rates by FRAP of GFP-tubulin; these experiments indicated that microtubules in the outer segment are long lived compared to the inner segment. The authors then addressed the molecular mechanisms that could contribute to the formation of the arrays of short microtubules in the outer segments by focusing on a microtubule severing enzyme, p60-L1, that is highly expressed in halteres. P60-L1 localized to outer segments, as observed with expression of a RFP-tagged p60-L1 construct. In functional tests, the authors found that two independent p60L1 mutants (one of them a bona fide null) exhibited defective flight, but NOT disruption of mechanically-evoked depolarization, indicating that mechanotransduction was intact, but the amplitude of the response was severely diminished. Mutants also exhibited defective outer segment structure and this correlated with loss of microtubules and diminished localization of the TRP channel NOMPC. The authors interpreted this
as a requirement for p60-L1 to generate the population of short microtubules required for normal assembly of the outer segment network by AMPLIFYING polymer levels. Ultrastructural analysis of the inner segment, however, revealed a distinct role for p60-L1 to prevent abnormal accumulation of microtubules, suggesting that, in this compartment, p60-L1 is surpassing overall polymerization. In vitro experiments revealed that recombinant p60-L1 possesses an ATP-dependent microtubule severing activity. The authors conclude their study with an analysis of p60-L1.patronin double mutants and present convincing data that the two proteins function cooperatively to promote microtubule polymer formation in a compartmentally-specific mechanism.

Overall, I find this paper to be convincing and the data of very high quality. The authors used most of appropriate controls (see below) and discussed their observations and model in a critical manner. I feel this study will be of broad interest to the reader ship of JCB, especially for readers interested in mechanosensation and ciliogenesis. I recommend for publication with a few minor revisions.

I understand the potential difficulty of conducting additional experiments given the restricted laboratory access we are all experiencing due to the COVID pandemic. However, I feel the behavioral (flight test) and electrophysiological data are the key functional results in this study. I felt the omission of positive controls for both of these sets of experiments made it difficult to evaluate the magnitude of the observed phenotypes. I would ask the authors to include additional data (Figures 3E,F) to show how loss-of-function of genes that 1) abrogated mechanosensation (perhaps NOMPC) and 2) compromised microtubule polymer levels in the outer segment (perhaps EB1, Mini spindles, DCX) affected flight and mechanically-evoked firing.

In the results section corresponding to localization of p60-L1, the authors don't clearly state they used RFP-p60-L1 to examine sub-cellular localization of the protein. This section (page 8, second paragraph) should be edited to clarify this point.

The authors have established several fly lines that will be of great use to the larger community of Drosophila labs (e.g. Patronin-RFP knock in lines).

The manuscript should be carefully proofread for grammatical errors (e.g. basis instead of bases).

I'd like to know where acetylated tubulin is by IF in relation to the microtubule networks they described by EM. Given it's role in mechanotransduction, I think this would be a valuable piece of data to round out the story.

Reviewer #3 (Comments to the Authors (Required)):

In this manuscript Sun et al use electron tomography combined with electrophysiology, genetics and in vitro assays to identify a katanin p60 isoform as important for controlling the architecture of the microtubule array in the fly campaniform mechanoreceptors. This is an interesting study and definitely of interest to the JCB readership. It is well-executed and provides exciting new insights into the role microtubule severing enzymes and the intimate connection between precise cytoskeletal architecture and the distribution of the NOMPC channel for optimal mechanosensitivity in these receptors. It also highlights why phenotypes for severing enzymes have been hard to characterize: they require high resolution reconstructions in dense microtubules arrays, which the ET now allows, albeit with considerable more work than light microscopy. That said, I have a few comments that I urge the authors to take into account.
General comments:

The use of the term "severase" in this manuscript is not appropriate. These enzymes have been referred to as "severing enzymes" for 30 years, including by their discoverers and it is not appropriate to now ad hoc change their name because the Howard lab published a recent paper in which inexplicably they decided to rename them. They appear as severing enzymes in the Alberts textbook and in all other papers in the literature. Out of consideration for the scientists who actually discovered them and named them and also to avoid confusion in the literature (and even increase the probability of finding this very manuscript through Pubmed searches) the term "severing enzyme" should be used throughout the manuscript.

I think the impact of the manuscript would be improved if the authors could get some help with the writing. There is odd use of language throughout the manuscript that unfortunately detracts from the clarity of the scientific arguments and data presented.

Specific comments:

1. Please indicate in figure legends the technique used to obtain data so that reader can easily access and evaluate it. This applies to all figures, but is especially important for the panels with ET microtubule reconstructions.
2. It is stated that katanin is highly expressed in these mechanosensors - can the authors provide an approximate concentration? And how does that compare with that in other cell types? And concentrations used in in vitro assays? Please also show some intermediate time points in the severing assay to show clear severing activity.
3. This is not required as additional data for this paper, but if known, can the authors include references on what gTurc distribution is in the inner and outer segments?
4. It seems to me that the microtubule persistence length argument regarding the importance of many short MTs to provide NompC anchoring sites vs longer, highly curved MTs is maybe a bit simplistic. Microtubules in platelets (~2micron diameter and well below the persistence length of MTs) are highly curved, so in principle you could have longer microtubules that are highly bent and provide sufficient anchoring sites for the mechanosensitive channels. It seems to me that there is a deeper meaning behind the microtubule architecture seen in the MO that likely has to do with how tension is stored and relayed in these structures.
5. The organization of the MO between the haltere and leg receptors is different -are the types of mechanical stimuli experienced by these receptors different? The fanned-out structure of the MO in the haltere receptor is quite striking and would possibly allow for a higher level of regularity to the NompC channel distribution? Can the authors comment on these differences?
6. The work of Srayko et al. should be cited/mentioned since it provides another example of electron tomography use (maybe the only other one in addition to this ms) to show that katanin can function in creating dense arrays of shorter microtubules (in that case in C. elegans meiosis for chromatin-based nucleation). This is especially appropriate given the statement the authors make on page 15 " However, due to the limited optical resolution, visualizing the dense microtubule networks where the severing activity is important remains challenging (18). Our 3D structural reconstruction at near-molecular resolution provides direct evidence to demonstrate the severing activity of katanin p60-L1 in shaping dense microtubule networks, thereby refining our understanding on the neuronal and ciliary function of microtubule severase."
7. When first mentioning katanin at the top of page 8 - a reasoning should be given why short microtubules should be associated with the activity of this protein ie it should be stated it is a microtubule severing enzymes and the original work that has shown katanin to be a microtubule...
severing enzymes by McNally, Vale and colleagues should be cited (McNally et al, Cell 1993).
8. When mentioning the known stabilizing role of Patronin for non-centrosomal MTs - please give citations here.
9. The idea that microtubule severing enzymes can be used to amplify microtubules was first proposed in 2006 (Roll-Mecak and Vale; Ribbeck and Mitchison).
10. The regular organization of the microtubules in the MO with respect to their polarity suggests a mechanism of organization after severing possibly dependent on a motor? Can the authors comment on this using the available literature of mutants with defects in mechanosensation?
JCB manuscript #202004184

Editor

Thank you for submitting your manuscript entitled "Dual Functions of Katanin p60-L1 in Sculpting the Cytoskeleton in Fly External Mecha

sensory Cilia". We are truly very sorry for the significant and unusual delay in processing the paper and sending our decision to you. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

As you will see, all of the reviewers found the work to be potentially of significant interest and the data of high quality. All had suggestions, most of which can be addressed by changes to the text. Reviewers #1 and #2 had some experimental suggestions for strengthening the mechanistic underpinnings of the behavioral phenotypes -- finding ways to address these would strengthen the work.

We thank the editors for handling our manuscript and offering the opportunity to submit the revision. We also thank three reviewers for their comments, which are constructive and helpful. Please see below our point-to-point responses.

Reviewer #1 (Comments to the Authors (Required)):

Living organisms need mechanosensation for survival and force perception. The role of cytoskeleton in mechanosensation is barely studied and remains elusive. In this manuscript, the authors reconstructed the near-molecular resolution structure of the microtubule based cytoskeleton in the sensory cilium of fly campaniform mechanoreceptors using electron tomography, and resolved two ordered microtubule arrays in the outer segment. To investigate how the microtubule structure is formed, the authors found the microtubule severing enzyme Kat-60L playing a dual role sculpting the microtubule structure in campaniform mechanoreceptors, which in turn regulates the mechanosensitivity of these receptors. This is an interesting study and there are a lot of nice data presented in this paper. I think it is suitable for JCB provided the authors can address a few issues.

Specific questions:
1. Katanins including Kat-60L1 have been shown to play an important role in neuron development such as axonal growth and dendrite pruning. How do the authors know the reduced mechanosensitivity of the haltere receptors in the kat-60L1 mutant is not due to general developmental/morphological defect of the receptors? In fact, kat-60L1BE6/PBac mutant larvae are also less sensitive to noxious heat (Reference 15). And here Fig 4E-F does show that the MO in the kat-60L1 mutant is somewhat structurally deformed. What is the mechanosensitivity of the mutant leg receptors that maintain a wild type-like structure?

We thank the reviewer for this question, which helps us to improve the rigor of this manuscript. Here are our thoughts.

(1) To strengthen our conclusion, we established a new assay to measure the mechanosensory responses of leg campaniform mechanoreceptors using in vivo calcium imaging. The mechanosensory responses in c01236/BE6 mutant is largely reduced, in consistence with the results
on haltere receptors. The new data are provided in Fig. 4 and the description has been added accordingly (p7, 1st paragraph).

2. The function of kat-60L1 has been implicated in regulating dendritic morphology (Stewart et al., 2012) and dendrite pruning (Lee et al., 2009) in fly da neurons. Given the main functions of kat-60L1 in dendrite, we measured the lengths of dendrite in leg campaniform receptors and the cross-sectional area of dendrite in haltere campaniform receptors. No significant difference was found between wild-type and the mutant, suggesting that there’re no major defects in the morphology of dendritic inner segment. The new data are provided in Fig. 4 and the description has been added accordingly (p11, 2nd paragraph).

3. Larval da neurons (e.g. class III and IV neurons) have more extensive dendritic arbors, and their molecular sensors (e.g. NompC in class III cells (Yan et al., 2013)) are thought to distribute all over their dendrites. Therefore, dendritic morphology determines the distribution of molecular sensors and, in turn, the mechanosensitivity. On the contrary, type I mechanoreceptors (e.g. campaniform mechanoreceptor) have a single dendrite and a specific site of mechanotransduction at the distal tip of the outer segment, i.e. the MO. A series of studies have shown that the molecular composition and ultrastructure of the MO are crucial for the sensory function of these mechanoreceptors. Therefore, the molecular and ultrastructural defects observed in the c01236/BE6 mutant that we presented in this manuscript should contribute to the defects in mechanosensation.

2. Kat-60L1 expression is not only found in mechanosensory neurons but also in CNS. How do the authors know the flight behavior defect results from the defect in sensory neurons? More specific driver other than the pan-neuronal driver elav-Gal4 is needed in the rescue experiment.

   We thank the reviewer for this suggestion. We performed additional experiments using a more specific driver for ciliated sensory neurons (dcx-emap-gal4). Using this driver line, the flight behavior defect is rescued to the similar level as using elav-gal4, suggesting that the mechanosensory defects contribute to the behavior phenotypes. The new data are provided in Fig. 4 and the texts are modified accordingly (p7, 1st paragraph). In addition, our electrophysiological recording and in vivo calcium imaging data further specify the functions of kat-60L1 in mechanosensory neurons.

3. The authors used FRAP assay to show that microtubule is stable in the outer segment in Fig 2F. Previous study from the Parrish lab showed a positive correlation between microtubule stability and Drosophila larval mechanosensitivity (Yan et al., 2018). As the authors think Kat-60L1 plays a role in regulating mechanosensitivity as well, is microtubule stability affected in the c01236/BE6 mutant?

   (1) We cannot measure FRAP in the c01236/BE6 mutant, because the mutant receptors showed very weak tubulin signal (close to background intensity) in the outer segment.

   (2) We think that the stability of microtubules is not likely to be changed in c01236/BE6 based on three lines of thoughts and evidence. First, microtubules in the outer segment show a relatively high level of acetylation (Liang et al., 2011). Tubulin acetylation is still present in c01236/BE6 (see below, Fig. r1). Second, kat-60L1 itself is a microtubule severing enzyme. Loss of kat-60L1 is not expected to decrease the stability of microtubules, which is likely provided by other factors,
such as acetylation and stabilizing MAPs. Third, Patronin (Fig. 6H) and DCX-EMAP (see below, Fig. r1), two known stabilizing MAPs for microtubules in the outer segment, are still correctly located in the outer segment in the c01236/BE6 mutant.

**Figure r1.** Acetylated microtubule and DCX-EMAP in the haltere campaniform mechanoreceptors in c01236/BE6. Left: haltere sample stained using an anti-acetylated tubulin antibody (T6793, Sigma) (Liang et al., 2011). Right: haltere sample stained using an anti-DCX-EMAP antibody (clone E08) (Liang et al., 2014). Scale bar: 10 μm.

4. If the outer segments of bristle and campaniform mechanoreceptors share a similar organization, why is NompC positive region reduced in the outer segment of campaniform mechanoreceptor while much expanded/elongated in that of bristle mechanoreceptor?

The outer segments of the sensory neurons in bristle and campaniform receptors contain a tubular body (proximal) and a mechanoreceptive organelle (distal), thereby sharing a similar organization. However, the extracellular structures in these two types of sensilla are different. In campaniform sensilla, the MO develops in a small space surrounded by other extracellular structures (Sun et al., PNAS, 2019). A dome-like cuticular structure, called cupola, overlays the distal end of the MO and prevents the MO from elongating further distally. In the kat-60L1 mutant, because microtubules are longer in length and fewer in number, the cytoskeleton in the MO cannot be well formed, which, in turn, results in a poorly-developed/smaller MO. In bristle cells, there is no cupola-like structure and the lumen space of the bristle shaft potentially allows the MO to elongate further distally (Keil, 1997), so that the NompC positive region can elongate into the bristle lumen in the kat-60L1 mutants where microtubules in the outer segment become much longer. Because of word limit, we have now moved these results into supplementary data (Fig. s5) and the corresponding texts are also modified to make this issue clearer (**p8, 3rd paragraph and the legend of Fig. s5**)

5. Is Kat-60L1 expressed in S2 cells? Could Kat-60L1 affect mechanosensitivity of S2 cells with ectopic expression of mechanosensitive channels like NompC? If there is no endogenous Kat-60L1, what happens if co-expressing NompC and Kat-60L1?

(1) According to our unpublished data and the high throughput expression data in Flybase, kat-60L1 has nearly no expression in S2 cells.

(2) Kat-60L1 is not expected to have a direct interaction with NompC because it does not co-localize with NompC *in vivo* and its absence does not affect the subcellular localization of
NompC. Furthermore, kat-60L1 is not likely to be a component in the mechanotransduction apparatus in fly mechanosensory cilia as its absence does not abolish mechanotransduction but reduces mechanosensitivity.

(3) Ectopic expression kat-60L1 largely disrupts the microtubule networks in S2 cells (Fig. r2), likely due to its severing activity. It was shown that microtubule destabilization drugs, such as nocodazole, largely abolish the mechanosensory current mediated by ectopically expressed NompC in S2 cells (Zhang, et al., Cell, 2015). Therefore, we think that ectopic over-expression of kat-60L1 in S2 cells would possibly change the gating behavior of ectopically expressed NompC in S2 cells, but more likely by altering the microtubule networks rather than directly regulating NompC itself.

**Figure r2.** Ectopic expression of RFP-kat-60L1-long disrupts the microtubule networks in S2 cells that stably express GFP-αtub84B (a stable cell line). Left: GFP-αtub84B. Middle: RFP-kat-60L1-long (vector: pARW). Right: merged image. Scale bar: 10 mm. Note that the cell expressing RFP-kat-60L1-long (white arrowhead) showed a much weaker microtubule signal in comparison to other non-transfected cells.

Minor questions:
1. A standard abbreviation of katanin p60-like 1 in flybase and other literatures is kat-60L1 instead of kat p60-L1. The authors should follow flybase.

   We thank the reviewer for pointing out this issue. We now use kat-60L1 (as listed in Flybase) throughout the manuscript.

2. Figure3F should have a negative control that is supposed to have no response (eg. nompC mutant).

   Added (Fig. 4).

**Reviewer #2 (Comments to the Authors (Required)):**

In this manuscript, the authors’ goal was to determine how the microtubule cytoskeleton contributes to mechanosensation using fly halter and leg campaniform mechanoreceptors as models. They began their study with an impressive set of experiments using use high-resolution tomographic EM to describe microtubule organization in the outer segments of these cells and identified two independent microtubule arrays that appear to be common to both cell types. Microtubules in the
outer segments appear to be extensively cross-linked with each other and with a basket of peripheral doublet microtubules by poorly-resolved electron-dense structures. They then used the minus end binding protein Patronin to establish that microtubules in the outer segments exhibit a uniform polarity with their plus ends distal to the cell bodies and this observation was further supported by imaging of EB1, a plus end marker. These analyses added further evidence to the existence of two disparate microtubule networks as the EB1 trajectories in the inner segments revealed microtubules in this compartment are oriented with their minus ends distal to the centers of the cells. The authors next examined microtubule turn-over rates by FRAP of GFP-tubulin; these experiments indicated that microtubules in the outer segment are long lived compared to the inner segment. The authors then addressed the molecular mechanisms that could contribute to the formation of the arrays of short microtubules in the outer segments by focusing on a microtubule severing enzyme, p60-L1, that is highly expressed in halteres. P60-L1 localized to outer segments, as observed with expression of a RFP-tagged p60-L1 construct. In functional tests, the authors found that two independent p60l1 mutants (one of them a bona fide null) exhibited defective flight, but NOT disruption of mechanically-evoked depolarization, indicating that mechanotransduction was intact, but the amplitude of the response was severely diminished. Mutants also exhibited defective outer segment structure and this correlated with loss of microtubules and diminished localization of the TRP channel NOMPC. The authors interpreted this as a requirement for p60-L1 to generate the population of short microtubules required for normal assembly of the outer segment network by AMPLIFYING polymer levels. Ultrastructural analysis of the inner segment, however, revealed a distinct role for p60-L1 to prevent abnormal accumulation of microtubules, suggesting that, in this compartment, p60-L1 is surpassing overall polymerization. In vitro experiments revealed that recombinant p60-L1 possesses an ATP-dependent microtubule severing activity. The authors conclude their study with an analysis of p60-l1;patronin double mutants and present convincing data that the two proteins function cooperatively to promote microtubule polymer formation in a compartmentally-specific mechanism.

Overall, I find this paper to be convincing and the data of very high quality. The authors used most of appropriate controls (see below) and discussed their observations and model in a critical manner. I feel this study will be of broad interest to the reader ship of JCB, especially for readers interested in mechanosensation and ciliogenesis. I recommend for publication with a few minor revisions.

I understand the potential difficulty of conducting additional experiments given the restricted laboratory access we are all experiencing due to the COVID pandemic. However, I feel the behavioral (flight test) and electrophysiological data are the key functional results in this study. I felt the omission of positive controls for both of these sets of experiments made it difficult to evaluate the magnitude of the observed phenotypes. I would ask the authors to include additional data (Figures 3E,F) to show how loss-of-function of genes that 1) abrogated mechanosensation (perhaps NOMPC) and 2) compromised microtubule polymer levels in the outer segment (perhaps EB1, Mini spindles, DCX) affected flight and mechanically-evoked firing.

We thank this reviewer for this advice. We have now modified this part as following:
In flight test, we have provided an addition rescue strain to specify the function of kat-60L1 in sensory neurons as requested by reviewer 1 and nompC3 (a null mutant) as a negative control with abrogated mechanotransduction as suggested by this reviewer. In addition, to our knowledge, the only other mutant known to have a compromised microtubule-based structures in the outer segment is a piggyBAC insertion mutant of dcx-emap (f02655 strain) (Bechstedt et al., 2010). However, according to our unpublished data, there are two issues with the f02655 strain. First, all our trials to rescue f02655 failed (including using cDNA, genome segment, etc.), suggesting that in addition to the effects caused by the loss of DCX-EMAP, there’re possibly other unknown reasons underlying the observed phenotypes in this strain. Second, the structural and molecular defects in f02655 have not been characterized to the level as we have done for kat-60L1 in the present work and the working mechanism of DCX-EMAP remains elusive. For these two reasons, we do not think that f02655 is a very good control for behavioral experiments. In fact, we have created a new KO mutant of dcx-emap that is genetically clean and can be functionally rescued. We hope to report our new findings on the functions and working mechanism of DCX-EMAP in a separate work.

Despite the abovementioned problems for behavior assays, it is clear that the outer segment of campaniform mechanoreceptors in f02655 mutants has structural defects (Bechstedt et al., 2010; Liang et al., 2014). Therefore, we think it is still reasonable to use it as a control in electrophysiological recording experiments on haltere campaniform mechanoreceptors. Therefore, we have now provided additional recording data on nompC3 and f02655 flies, both as controls (Fig. 4). These new data strengthen the point that in the kat-60L1 mutants, mechanotransduction is intact but mechanosensitivity is reduced. The corresponding texts are modified accordingly (p7, 1st paragraph).

In the results section corresponding to localization of p60-L1, the authors don’t clearly state they used RFP-p60-L1 to examine sub-cellular localization of the protein. This section (page 8, second paragraph) should be edited to clarify this point.

We have now modified this part (p6, 4th paragraph and legend of Fig. 4).

The authors have established several fly lines that will be of great use to the larger community of Drosophila labs (e.g. Patronin-RFP knock in lines).

We would be very happy to share all lines that we created.

The manuscript should be carefully proofread for grammatical errors (e.g. basis instead of bases).

We have now tried our best to proofread the manuscript.

I’d like to know where acetylated tubulin is by IF in relation to the microtubule networks they described by EM. Given it’s role in mechanotransduction, I think this would be a valuable piece of data to round out the story.

The microtubules in the outer segment of haltere campaniform mechanoreceptors showed a relatively high level of acetylation (in Figure 2D of Liang, et al., 2011, Cytoskeleton,
We agree with the reviewer that the modification state, especially acetylation due to its role in mechanosensation (Yan et al., 2018), is one of the basic properties of microtubules in the outer segment, so we have now edited the text to make this point clear in the introduction (p2, 2nd paragraph).

Reviewer #3 (Comments to the Authors (Required)):

In this manuscript Sun et al use electron tomography combined with electrophysiology, genetics and in vitro assays to identify a katanin p60 isoform as important for controlling the architecture of the microtubule array in the fly campaniform mechanoreceptors. This is an interesting study and definitely of interest to the JCB readership. It is well-executed and provides exciting new insights into the role microtubule severing enzymes and the intimate connection between precise cytoskeletal architecture and the distribution of the NOMPC channel for optimal mechanosensitivity in these receptors. It also highlights why phenotypes for severing enzymes have been hard to characterize: they require high resolution reconstructions in dense microtubules arrays, which the ET now allows, albeit with considerable more work than light microscopy. That said, I have a few comments that I urge the authors to take into account.

General comments:
The use of the term "severase" in this manuscript is not appropriate. These enzymes have been referred to as "severing enzymes" for 30 years, including by their discoverers and it is not appropriate to now ad hoc change their name because the Howard lab published a recent paper in which inexplicably they decided to rename them. They appear as severing enzymes in the Alberts textbook and in all other papers in the literature. Out of consideration for the scientists who actually discovered them and named them and also to avoid confusion in the literature (and even increase the probability of finding this very manuscript through Pubmed searches) the term "severing enzyme" should be used throughout the manuscript.

We thank the reviewer for all the comments and suggestions. They are very helpful to place the present work in the historical context of studying katanin family proteins. More specifically, we now use “severing enzyme” throughout the manuscript.

I think the impact of the manuscript would be improved if the authors could get some help with the writing. There is odd use of language throughout the manuscript that unfortunately detracts from the clarity of the scientific arguments and data presented.

We have now tried our best to proofread the manuscript.

Specific comments:

1. Please indicate in figure legends the technique used to obtain data so that reader can easily access and evaluate it. This applies to all figures, but is especially important for the panels with ET microtubule reconstructions.
We thank the reviewer for this suggestion. We have now indicated “ET slice images” for all panels with ET reconstructions.

2. It is stated that katanin is highly expressed in these mechanosensors - can the authors provide an approximate concentration? And how does that compare with that in other cell types? And concentrations used in in vitro assays? Please also show some intermediate time points in the severing assay to show clear severing activity.

(1) We concluded that kat-60L1 highly expresses in the type I mechanoreceptors by examining the expression pattern of kat-60L1 using the promoter-gal4 system (Fig. 4B and Fig. s3).

(2) Our unpublished RNA-sequencing data show that the expression level of kat-60L1-long in haltere pedicel (containing ciliated sensory neurons) is ~20 times higher than that in haltere capitellum (containing mostly epidermal cells) and ~42 times higher than that in fly brain tissue (neuronal cells). This analysis supports the conclusion that kat-60L1 highly expresses in these mechanosensory neurons. It is difficult to precisely quantify the absolute concentration of kat-60L1 in these cells but we estimate the concentration as following. The physiological concentration of katanin in Hela was estimated to be around 20-50 nM (Vemu et al., 2018) and 50-100 nM in neuronal tissues (Solowska et al., 2008). Given that the expression of kat-60L1 in fly ciliated mechanoreceptors is much higher than an average neuronal cell (our RNA-sequencing data) and the signal of kat-60L1 is localized in these mechanoreceptors, we estimate that the local concentration of kat-60L1 at the basal body and in the outer segment is at least at the order of a few hundreds of nM, similar to the concentration that was used in our severing assays (300 nM).

(3) Following the suggestion of the reviewer, we have now added intermediate time points in panels for the severing assay (Fig. s3, panel G and H).

3. This is not required as additional data for this paper, but if known, can the authors include references on what gTurc distribution is in the inner and outer segments?

The molecular organization of microtubules in fly type I mechanoreceptors has not been well studied before the present work. Using a GFP-γ-tubulin23C fly strain, we found that there is an enriched signal of γ-tubulin around the basal body (arrow 1) and some weak signals along the dendritic membrane in the inner segment (arrow 2) (Fig. r3). No prominent signal was detected in the outer segment (Fig. r3). We hypothesize that γ-tubulin may be involved in organizing the “minus-end-distal” microtubules in the inner segment, but based on the localization, it doesn’t seem that γ-tubulin would play major roles in organizing microtubules in the outer segment. We think that more studies are required to further clarify the role of γ-tubulin in organizing the microtubules in the inner and outer segments.
4. It seems to me that the microtubule persistence length argument regarding the importance of many short MTs to provide NompC anchoring sites vs longer, highly curved MTs is maybe a bit simplistic. Microtubules in platelets (~2micron diameter and well below the persistence length of MTs) are highly curved, so in principle you could have longer microtubules that are highly bent and provide sufficient anchoring sites for the mechanosensitive channels. It seems to me that there is a deeper meaning behind the microtubule architecture seen in the MO that likely has to do with how tension is stored and relayed in these structures.

We thank the reviewer for this comment which is truly thoughtful. In fact, we demonstrated in our previous work that the MO indeed withstands resting tensions for optimal mechanosensitivity. After re-considering this issue, we simplified the discussion on this point (also due to the word limit), but made it clear that short microtubules facilitate the structural-mechanical design of the MO, for example by providing adequate anchoring sites for NompC force-sensitive channels or storing tensions for optimal sensitivity (p10, 2nd paragraph).

5. The organization of the MO between the haltere and leg receptors is different - are the types of mechanical stimuli experienced by these receptors different? The fanned-out structure of the MO in the haltere receptor is quite striking and would possibly allow for a higher level of regularity to the NompC channel distribution? Can the authors comment on these differences?

Based on literatures and our own ultrastructure studies (Dickinson, 1992; Sun et al., 2019), it is known that haltere receptors are excited by curvature change in cuticular structures when halteres are beating, and they show clear directional sensitivity. We demonstrated in our previous work that the fan-shaped morphology and the intracellular organization of the MO support the directional sensitivity of haltere receptors (Sun et al., 2019). In particular, the two line arrays of microtubules in the MO are key in organizing the regular array of NompC channels on the MO membrane. Furthermore, NompC channels in the MO are anchored to the microtubules and by doing so, they are oriented in the way that the ankyrin helix of NompC, thought to be the gating spring, can be elongated or shortened in response to stimulating forces.
On the contrary, there are very few studies on the sensory functions of leg receptors, probably because they are isolated receptors and more difficult to study. We think that leg receptors respond to cuticle deformation that is generated during walking or jumping. However, because the overall organization of the entire sensilla (including all extracellular structures) has not been reconstructed, it is not yet clear how the macroscopic cuticular deformation or muscular activity can be converted into the proximal stimuli of the sensory neurons. Furthermore, we think NompC channels in the MO of leg receptors likely also form regular arrays because the microtubules closed to membrane are organized (based on our ET data). However, to directly visualize these arrays, we would need to further improve our sample preparation skill to obtain more cross sections.

6. The work of Srayko et al. should be cited/mentioned since it provides another example of electron tomography use (maybe the only other one in addition to this ms) to show that katanin can function in creating dense arrays of shorter microtubules (in that case in C. elegans meiosis for chromatin-based nucleation). This is especially appropriate given the statement the authors make on page 15 "However, due to the limited optical resolution, visualizing the dense microtubule networks where the severing activity is important remains challenging (18). Our 3D structural reconstruction at near-molecular resolution provides direct evidence to demonstrate the severing activity of katanin p60-L1 in shaping dense microtubule networks, thereby refining our understanding on the neuronal and ciliary function of microtubule severase."

We thank the reviewer for this suggestion. We have now added this reference and edited the text accordingly (p10, last paragraph).

7. When first mentioning katanin at the top of page 8 - a reasoning should be given why short microtubules should be associated with the activity of this protein ie it should be stated it is a microtubule severing enzymes and the original work that has shown katanin to be a microtubule severing enzymes by McNally, Vale and colleagues should be cited (McNally et al, Cell 1993).

We thank the reviewer for this suggestion. We have now added the reference (p6, 3rd paragraph).

8. When mentioning the known stabilizing role of Patronin for non-centrosomal MTs - please give citations here.

We have now added the references (p5, 1st paragraph).

9. The idea that microtubule severing enzymes can be used to amplify microtubules was first proposed in 2006 (Roll-Mecak and Vale; Ribbeck and Mitchison).

We have now added the references (p10, last paragraph).

10. The regular organization of the microtubules in the MO with respect to their polarity suggests a mechanism of organization after severing possibly dependent on a motor? Can the authors comment on this using the available literature of mutants with defects in mechanosensation?
We agree with the reviewer that there should be a mechanism to organize the microtubules into an array in the outer segment. Indeed, a motor-dependent mechanism is attractive. However, to our knowledge, there’re no literatures available on any motor mutants that show structural and functional defects in this type of fly mechanoreceptors. There are certainly more questions awaiting to be answered in this model cell. In this view, I think the present study opens the gate and provides the basis to dissect the molecular basis of how this microtubule array is formed. Due to the word limit, we could not further discuss all these issues in the current manuscript, but hope to find the answer to these questions in our future studies.

Reference

Bechstedt, S., J.T. Albert, D.P. Kreil, T. Muller-Reichert, M.C. Gopfert, and J. Howard. 2010. A doublecortin containing microtubule-associated protein is implicated in mechanotransduction in Drosophila sensory cilia. Nat Commun. 1:11.

Dickinson, M.H. 1992. Directional Sensitivity and Mechanical Coupling Dynamics of Campaniform Sensilla During Chordwise Deformations of the Fly Wing. Journal of Experimental Biology. 169:221-233.

Keil, T.A. 1997. Functional morphology of insect mechanoreceptors. Microsce Res Tech. 39:506-531.

Lee, H.H., L.Y. Jan, and Y.N. Jan. 2009. Drosophila IKK-related kinase Ik2 and Katanin p60-like I regulate dendrite pruning of sensory neuron during metamorphosis. Proc Natl Acad Sci U S A. 106:6363-6368.

Liang, X., J. Madrid, and J. Howard. 2014. The microtubule-based cytoskeleton is a component of a mechanical signaling pathway in fly campaniform receptors. Biophys J. 107:2767-2774.

Liang, X., J. Madrid, H.S. Saleh, and J. Howard. 2011. NOMPC, a member of the TRP channel family, localizes to the tubular body and distal cilium of Drosophila campaniform and chordotonal receptor cells. Cytoskeleton (Hoboken). 68:1-7.

Solowska, J.M., G. Morfini, A. Falnikar, B.T. Himes, S.T. Brady, D. Huang, and P.W. Baas. 2008. Quantitative and functional analyses of spastin in the nervous system: implications for hereditary spastic paraplegia. J Neurosci. 28:2147-2157.

Stewart, A., A. Tsoubouchi, M.M. Rolls, W.D. Tracey, and N.T. Sherwood. 2012. Katanin p60-like1 promotes microtubule growth and terminal dendrite stability in the larval class IV sensory neurons of Drosophila. J Neurosci. 32:11631-11642.

Sun, L., Y. Gao, J. He, L. Cui, J. Meissner, J.M. Verbavatz, B. Li, X. Feng, and X. Liang. 2019. Ultrastructural organization of NompC in the mechanonocceptive organelle of Drosophila campaniform mechanoreceptors. Proc Natl Acad Sci U S A. 116:7343-7352.

Vemu, A., E. Szczesna, E.A. Zehr, J.O. Spector, N. Grigorieff, A.M. Deaconescu, and A. Roll-Mecak. 2018. Severing enzymes amplify microtubule arrays through lattice GTP-tubulin incorporation. Science. 361.

Yan, C., F. Wang, Y. Peng, C.R. Williams, B. Jenkins, J. Wildonger, H.J. Kim, J.B. Perr, J.C. Vaughan, M.E. Kern, M.R. Falvo, E.T. O’Brien, 3rd, R. Superfine, J.C. Tuthill, Y. Xiang, S.L. Rogers, and J.Z. Parrish. 2018. Microtubule Acetylation Is Required for Mechanosensation in Drosophila. Cell Rep. 25:1051-1065 e1056.
Yan, Z., W. Zhang, Y. He, D. Gorczyca, Y. Xiang, L.E. Cheng, S. Meltzer, L.Y. Jan, and Y.N. Jan. 2013. Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. Nature. 493:221-225.
October 13, 2020

RE: JCB Manuscript #202004184R

Prof. Xin Liang
Tsinghua University
Room E118
Medical School, Tsinghua University
Beijing 100084
China

Dear Prof. Liang,

Thank you for submitting your revised manuscript entitled "Dual Functions of Kat-60L1 in Sculpting the Cytoskeleton in Fly External Mechanosensory Cilia". Thank you for your efforts revising the work - both reviewers enthusiastically recommend publication. We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

1) Titles, eTOC: Please consider the following revision suggestions aimed at increasing the accessibility of the work for a broad audience and non-experts.

Title: Dual Functions of Kat-60L1 in Sculpting the Cytoskeleton in Mechanosensory Cilia
or Kat-60L1 sculpts the cytoskeleton in mechanosensory cilia

Running title (50 characters max, including spaces): Dual roles of kat-60L1 in mechanosensory cilia

eTOC summary: A 40-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.
- Please include a summary statement on the title page of the resubmission. It should start with "First author name(s) et al..." to match our preferred style.
Suggested revision to match this style:

In mechanoreceptor cells, specialized cytoskeletons are formed at the site of mechanotransduction. Sun et al reconstruct the microtubule-based cytoskeleton in fly campaniform mechanosensory cilia using electron microscopic tomography and establish dual functions for kat-60L1 in shaping the cytoskeleton.

2) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to 3D, S1G (magnifications)
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis. Please add molecular weight with unit labels on the following panels: 4C

3) Statistical analysis: Error bars on graphic representations of numerical data must be clearly
described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please indicate n/sample size/how many experiments the data are representative of: 2F, 4EGJ

4) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts. - Please provide database IDs for all fly lines (e.g., FlyBase or BDSC) *even if gifted by other investigators or described in other published work* or if not available, please describe their basic genetic features *even if gifted by other investigators or described in other published work* - Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
  a. Make and model of microscope
  b. Type, magnification, and numerical aperture of the objective lenses
  c. Temperature
  d. Imaging medium
  e. Fluorochromes
  f. Camera make and model
  g. Acquisition software
  h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstructions, surface or volume rendering, gamma adjustments, etc.).

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and video files: See our detailed guidelines for preparing your production-ready images, https://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

**It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication.**
Please ensure that you have access to all original data images prior to final submission.**

**The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.**

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g., if you cannot retrieve necessary files from your laboratory), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Mark Peifer, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The authors have done a very nice job addressing my comments. I support the publication of this paper in JCB.

Reviewer #2 (Comments to the Authors (Required)):

The authors have addressed my comments nicely. I would advocate for publication in JCB.