KIF14 controls ciliogenesis via regulation of Aurora A and is important for Hedgehog signaling

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May 30, 2019

Re: JCB manuscript #201904107

Dr. Lukas Cajanek
Department of Histology and Embryology, Masaryk University, Faculty of Medicine, Brno, Czech Republic
Kamenice 3
Brno, Česká Republika 62500
Czech Republic

Dear Dr. Cajanek,

Thank you for submitting your manuscript entitled "KIF14 controls ciliogenesis via regulation of Aurora A in human cells". The manuscript has been evaluated by expert reviewers, whose reports are appended below. Unfortunately, after an assessment of the reviewer feedback, our editorial decision is against publication in JCB.

You will see that there is interest in the proposed role for KIF14 in regulating ciliogenesis but some concern noted about the degree of mechanistic insight from Reviewer #2. More importantly, Reviewer #3 raises a major issue with the proposal that KIF14 has no effect on cytokinesis but regulates ciliogenesis, as both these claims contradict earlier work in zebrafish and two cell lines (including a similar knockdown approach in RPE cells). Reviewer #3's concerns about the disparity with in vivo data would have to be addressed very rigorously and it is not clear to us how feasible this may be, or how confident we could be about the potential for publication after revision. In addition to this issue, the technical comments raised by Reviewer #1 and #3 would have to be addressed in detail and the work more clearly discussed in the context of prior literature as recommended by Reviewer #1. If you do wish us to consider resubmission, we would recommend that you provide a revision plan at a very early stage outlining in detail how you envisage addressing Reviewer #3's comments about the disparity with earlier studies to avoid spending time on experimental revisions that may not be satisfactory for re-review. If you wish to expedite publication of the current data, it may be best to pursue publication at another journal.

If you would like to resubmit this work to JCB, please contact the journal office to discuss an appeal of this decision or you may submit an appeal directly through our manuscript submission system. Please note that priority and novelty would be reassessed at resubmission.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss the reviewer comments further once you've had a chance to consider the points raised in this letter. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

John Wallingford, Ph.D.

Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

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

Pejskova et al show that the mitotic kinesin KIF14 functions in mammalian cell lines to regulate ciliogenesis, ciliary length, and Shh signaling. Human patients with homozygous recessive mutations in KIF14 have mild to very severe microcephaly, a ciliopathy-like phenotype. This manuscript clearly establishes a role for KIF14 in primary cilia of cultured mammalian cells.
Kinesin-3 motors act in ciliary processes in C. elegans and mammals (KLP-6 - work by Barr lab and KIF13b - work by Christensen and Pedersen labs). That KIF14 is a member of this family and is a newly discovered ciliary kinesin is exciting and will be appealing to the JCB readership. Authors show that KIF14 localizes to the primary cilium (Figure 5), regulates ciliogenesis/ciliary length (Figure 1), influences Shh-induced accumulation of Smoothened (SMO) and GLI1 expression (Figure 2), and is required for localization of select basal body and ciliary components (Figure 3). Furthermore, authors go onto show that KIF14 interactor Citron Rho-interacting kinase (Figure 4, including CIT, KIF14 and pharmacological manipulations) also impair ciliogenesis and that Aurora A acts downstream of KIF14 with respect to cilia formation (Figure 6, AURA inhibitor TCS7010 rescues KIF14 depletion ciliary phenotype).
However, the significance and impact of the manuscript as written and organized is not obvious. This could be easily rectified with rewriting and reorganization (see first paragraph for suggested order of Figures/data presentation). With respect to the former, authors provide no background on what is known about kinesins within the mammalian primary cilium or what is know about kinesins in regulating IFT in any system. Authors do not discuss the kinesin family to which KIF14 belongs. Buried in Supplemental figure 5A, I inferred that KIF14 is a kinesin-3 member, based on its FHA domain. From here I read the nice review article by authors Reilly and Benmerah (2019) to gain the necessary background to put this manuscript in a big picture context.
The title of the manuscript "KIF14 controls ciliogenesis via regulation of Aurora A in human cells" implies a global function in all human cilia. Schou et al Nature Communications (2015) show that Kif13b is highly upregulated in serum starved NIH3T3 cells while Kif14 is downregulated (Figure 1A). This suggests that Kif14 may play cell-specific roles in cilia, which would be consistent with rodent and human Kif14 mutant phenotypes. Would authors please address this possibility?
Specific comments:
Manuscript would benefit from editing for grammar
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Experiment in Figure 5 is poorly explained in the manuscript. Did authors actually measure KIF14 levels after pharmacological manipulations or is this a prediction?
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Figure 5: structure/localization studies show that the motor domain is sufficient to target KIF14 to cilia. Do these truncated GFP-KIF14 constructs have any dominant effects? In 5I, Cilia look longer

and misshapen when GFP-KIF14(1-708) is expressed compared to the shorter cilia of KIF14(1-356) and (800-1648). This should be addressed.
The rationale for using the missense and human disease KIF14 mutants should be better explained and more thoughtfully interpreted. How does the motor dead R364C KIF14 localize to cilia? Does this imply transport to cilia via another motor? How does the T456M mutation affect KIF14? Why would truncated mutants localize to the ciliary tip whereas KIF14(1-708) is evenly distributed along the primary cilium

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

This is an interesting paper that presents an unexpected ciliogenesis requirement for KIF14, a motor protein generally thought of as required for cytokinesis, although in the hands of the authors, this effect is not obvious. The authors present data that clearly indicates the requirement of KIF14 in ciliogenesis and the growth of cilia. This appears to be mediated through an affect on the subdistal appendages of the basal bodies. Desptie failing to see a clear involvement of KIF14 in cytokinesis, the authors do find that knockdown of the cytokinesis regulator, Citron kinase, also has a ciliogenesis phenotype.
The question is - what does KIF14 actually do in ciliogenesis. The authors are able to show its association with primary cilia and that KIF14 knockdown leads to activation of the cilia resorption pathway apparently via Aurora A. However, at the end of the paper, we are still left with an uncertainly about mechanism. For this reason, I do not believe that paper has reached the stage at which publication in JCB is appropriate although it may find a home in other cell biology journals.

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

In this manuscript, Pejskova et al conducted loss-of-function studies on the motor protein KIF14 in RPE1 cells. In contrast to the known function of KIF14 in mitosis/cytokinesis (demonstrated previously by both in vivo and in vitro studies), the authors here found that loss of KIF14 in RPE1 cells has no/little impacts on cytokinesis/mitosis, but severely abolishes ciliogenesis instead. They showed that ciliogenesis defects are likely due to the loss of KIF14's function in interphase rather than mitosis, and that overexpressed KIF14 can localize to the ciliary compartment. Moreover, the authors demonstrated that in response to KIF14 knockdown, aurora A kinase (AURA), an enzyme known to promote cilia disassembly, is ectopically activated at the centrosome, and that inactivation of AURA can fully rescue the ciliogenesis defect, restoring both cilia growth and length in KIF14-depleted cells. Together, the authors conclude that KIF14 has a novel role in promoting ciliogenesis through regulation of the AURA kinase activity.

Major concern:
1. The function of KIF14 in mitosis, including chromosome alignment/segregation and cytokinesis, has been well demonstrated previously through both in vivo and in vitro experiments, including in Drosophila, mice, zebrafish, human patients, and several tissue culture cell lines (e.g. HeLa cells_Gruneberg el al, 2006; IMCD3 & RPE1 cells_ Reilly et al, 2018). In some of the cellular studies, ciliogenesis was specifically examined (e.g. in KIF14-/- knockout IMCD3 cells or RPE1 cells where KIF14 was depleted by siRNA), but no defect in cilia assembly was found in any case (Reilly et al, 2018). In vivo, mutation of KIF14 in mice or human was shown to cause microcephaly, and at least in human, cytokinesis failure was indeed seen in patient cells, which was believed to be the cellular defect driving microcephaly (Moawia A el al, 2017). In most cases, microcephaly is a result of stress responses that react to defects in cellular processes such as DNA replication and mitosis. Lack of

ciliogenesis per se during brain development does not cause microcephaly.

The KIF14 phenotype described in the current manuscript, i.e. cilia defects but no mitotic stress, are largely inconsistent with these previous studies. I notice that the authors tried to discuss this issue in the discussion, but it is not exactly to the point, so I am not sure what to make of it, or what data to believe (although in vivo data is often more reliable). I think the authors need to experimentally reconcile these very serious discrepancies (see below).

Another note: Some cells (including RPE1 cells) can overcome the defects in cytokinetic abscission, as they often migrate vigorously and can physically break the bridges. Lack of binuclear cells does not mean that cytokinesis or mitosis is perfect.

2a. All data indicates that KIF14 is important for chromosome alignment/segregation or cytokinesis. Loss of KIF14 should therefore generate some forms/degrees of mitotic stress or insult to the cell. Even though a cytokinesis defect was not seen in this study, the authors cannot simply assume that their KIF14-depleted RPE1 cells are free of mitotic stress/insult. For example, is there a mild mitotic delay in response to KIF14 knockdown in RPE1 cells (should be)? If yes, does the mitotic delay correlate with the ciliogenesis defect seen in the coming G1 phase? Live-cell imaging with some correlated studies should work.

2b. The approach used in Figure 5 experiments is confusing; it is not clear to me how the authors can be sure about the protein level of KIF14 in each condition (mRNA level is not a direct indicator), especially when these cells are not under the same proliferation condition where we can assume all cells have the same protein turnover rate for KIF14. I have not seen this type of approach before, and I am not sure it is valid/flawless for the purpose.

2c. Most of mitotic stresses can lead to various degrees of p53 activation, which can globally alter the physiology of the stressed cell after mitosis. An interesting correlation that supports this notion is that IMCD3 cells are p53 compromised (SV40 transformed), and that IMCD3 can grow normal cilia in the absence of KIF14 (Reilly et al, 2018). My suggestion is that perhaps the authors can repeat the KIF14 knockdown experiment in p53-/- RPE1 cells that grow cilia, and check if cilia assembly occurs normally compared to control RNAi.

3. An alternative way to test the role of KIF14 specifically in interphase is to perform the rescue experiment specifically in G0-arrested cells, i.e. KIF14-depleted RPE1 cells are serum starved first, followed by expression of RNAi-resistant KIF14 (using lentivirus that carries the RNAi-resistant KIF14 expression construct). I would be very careful with the cell line carrying the Tet-On system, as the TRE promoter is always leaky.

4. The localization of the endogenous KIF14 during ciliogenesis should be examined, as over-expression can often cause artifacts or ectopic localizations, given that KIF14 is a MT binding protein and cilia are made of MT.

1st Revision - Authors' Response to Reviewers: December 20, 2019

Dear editor,

Please find attached our rebuttal letter in response to the reviewers' comments on manuscript "KIF14 controls ciliogenesis via regulation of Aurora A and is important for Hedgehog signaling" by Petra Pejskova et al., submitted previously to *Journal of Cell Biology* (manuscript 201904107).

First of all, we would like to thank all reviewers for their constructive comments and suggestions. I believe we have now addressed all major issues related to the reviewers' comments. In the revised manuscript we have included two new main figures and several additional panels to provide more additional evidence for our conclusions. Specifically, we have included new experiments as well as clarifications in the text to address the criticism of Reviewer#3 regarding discrepancies with previously published work on KIF14. We have also reorganized data and added new experiments to Figure 5, which previously raised concern from both Reviewer #1 and #3. In addition, we have included several additional experiments to fulfill the request of Reviewer #2 for more mechanistic insight. We also edited the manuscript accordingly in the Introduction and the Discussion, to relate our work better to the current literature and to improve overall clarity of our argumentation.

In sum, our data identify KIF14 as novel regulator of primary cilia formation and function. We demonstrate KIF14 has two separate roles in relation to PCs. The first one is related to PCs formation and is mediated via Aurora A. The second function of KIF14, which does not depend on Aurora A activity, is to ensure correct response of PCs to the HH pathway activation.

We sincerely hope you find our revised manuscript sufficiently improved for publishing in *Journal of Cell Biology.*

Looking forward to hearing from you,

On behalf of the authors,

Lukas Cajanek, PhD.

Dear Dr. Cajanek,

Thank you for submitting your manuscript entitled "KIF14 controls ciliogenesis via regulation of Aurora A in human cells". The manuscript has been evaluated by expert reviewers, whose reports are appended below. Unfortunately, after an assessment of the reviewer feedback, our editorial decision is against publication in JCB.

You will see that there is interest in the proposed role for KIF14 in regulating ciliogenesis but some concern noted about the degree of mechanistic insight from Reviewer #2. More importantly, Reviewer #3 raises a major issue with the proposal that KIF14 has no effect on cytokinesis but regulates ciliogenesis, as both these claims contradict earlier work in zebrafish and two cell lines (including a similar knockdown approach in RPE cells). Reviewer #3's concerns about the disparity with in vivo data would have to be addressed very rigorously and it is not clear to us how feasible this may be, or how confident we could be about the potential for publication after revision. In addition to this issue, the technical comments raised by Reviewer #1 and #3 would have to be addressed in detail and the work more clearly discussed in the context of prior literature as recommended by Reviewer #1. If you do wish us to consider resubmission, we would recommend that you provide a revision plan at a very early stage outlining in detail how you envisage addressing Reviewer #3's comments about the disparity with earlier studies to avoid spending time on experimental revisions that may not be satisfactory for re-review. If you wish to expedite publication of the current data, it may be best to pursue publication at another journal.

If you would like to resubmit this work to JCB, please contact the journal office to discuss an appeal of this decision or you may submit an appeal directly through our manuscript submission system. Please note that priority and novelty would be reassessed at resubmission.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss the reviewer comments further once you've had a chance to consider the points raised in this letter. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

John Wallingford, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Point by point reply to the comments:

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

Pejskova et al show that the mitotic kinesin KIF14 functions in mammalian cell lines to regulate ciliogenesis, ciliary length, and Shh signaling. Human patients with homozygous recessive mutations in KIF14 have mild to very severe microcephaly, a ciliopathy-like phenotype. This manuscript clearly establishes a role for KIF14 in primary cilia of cultured mammalian cells. Kinesin-3 motors act in ciliary processes in C. elegans and mammals (KLP-6 - work by Barr lab and KIF13b - work by Christensen and Pedersen labs). That KIF14 is a member of this family and is a newly discovered ciliary kinesin is exciting and will be appealing to the JCB readership. Authors show that KIF14 localizes to the primary cilium (Figure 5), regulates ciliogenesis/ciliary length (Figure 1), influences Shh-induced accumulation of Smoothened (SMO) and GLI1 expression (Figure 2), and is required for localization of select basal body and ciliary components (Figure 3). Furthermore, authors go onto show that KIF14 interactor Citron Rho-interacting kinase (Figure 4, including CIT, KIF14 and pharmacological manipulations) also impair ciliogenesis and that Aurora A acts downstream of KIF14 with respect to cilia formation (Figure 6, AURA inhibitor TCS7010 rescues KIF14 depletion ciliary phenotype).
However, the significance and impact of the manuscript as written and organized is not obvious. This could be easily rectified with rewriting and reorganization (see first paragraph for suggested order of Figures/data presentation).

We would like to thank this reviewer for suggestions regarding flow of the data and we agree it makes sense to put the data on KIF14 localization earlier in the manuscript. In this regard we moved the original KIF14 localization data to Fig. 2 and added new data on localization of the endogenous KIF14 (Fig. 2C).

With respect to the former, authors provide no background on what is known about kinesins within the mammalian primary cilium or what is know about kinesins in regulating IFT in any system. Authors do not discuss the kinesin family to which KIF14 belongs. Buried in Supplemental figure 5A, I inferred that KIF14 is a kinesin-3 member, based on its FHA domain. From here I read the nice review article by authors Reilly and Benmerah (2019) to gain the necessary background to put this manuscript in a big picture context.

To provide more background information about KIF14 and kinesins in general, we have included following paragraph in the Introduction (page 5-6):

(…) Here we report on a new role of kinesin KIF14 in PC formation in human cells. Kinesins are molecular motors, able to bind microtubules through their motor domain, and involved in various aspects of intracellular transport, including ciliogenesis (Hirokawa et al., 2009; Reilly and Benmerah, 2019; Silverman and Leroux, 2009). They may serve different functions in PC. While kinesin II complex is important to drive anterograde transport of IFT particles from base of the cilium to its tip (Cole et al., 1998; Marszalek et al., 1999; Morris and Scholey, 1997), KIF7 seems to organize HH signaling platform in the tip of PC (Endoh-Yamagami et al., 2009; He et al., 2014), and KIF24 has been proposed to negatively regulate early steps of PC formation (Kobayashi et al., 2011) as well as to mediate cilium disassembly (Kim et al., 2015). KIF14, a member of kinesin-3 subfamily, has been previously implicated in regulation of chromosome segregation (Zhu et al., 2005), cytokinesis and midbody formation (Bassi et al., 2013; Carleton et al., 2006; Gruneberg et al., 2006; Moawia et al., 2017; Ohkura et al., 1997). Interestingly, recent reports have suggested a possible link between KIF14

mutations and ciliopathies (Filges et al., 2014; Makrythanasis et al., 2018; Reilly et al., 2018). Yet no evidence that KIF14 is involved in ciliogenesis was provided. (…)

The title of the manuscript "KIF14 controls ciliogenesis via regulation of Aurora A in human cells" implies a global function in all human cilia. Schou et al Nature Communications (2015) show that Kif13b is highly upregulated in serum starved NIH3T3 cells while Kif14 is downregulated (Figure 1A). This suggests that Kif14 may play cell-specific roles in cilia, which would be consistent with rodent and human Kif14 mutant phenotypes. Would authors please address this possibility?

We are aware of reports showing that KIF14 expression peaks during mitosis (Carleton et al., 2006), (Schou et al., 2017). In fact, we observed similar behavior also in our models (data not shown). That, however in our opinion does not exclude a role for KIF14 in PC formation. Of note, ciliogenesis – regulating basal body proteins have been recently shown to be present in a cell at very low levels (Bauer et al, 2016, EMBO J). In our originally submitted manuscript, we observed PC – related phenotypes following KIF14 RNAi in two human cell lines – RPE-1 and nHDF line of fibroblasts. To address the point raised, we have now included data on KIF14 RNAi in additional cell lines, namely human embryonic stem cells (hESCs; Fig. S1N-P), human induced pluripotent stem cells (hiPSCs; Fig. S1Q-S), and murine lines IMCD3 (Fig.S1T-V). Interestingly, we observed similar phenotypes as before (reduced PC formation, formed PC typically shorter) in all these lines. Based on these data we argue that the function of KIF14 in PC formation is not restricted to one particular cell type. That being said, this does not formally exclude that KIf14 plays a context dependent role *in vivo*, which we also state in the Discussion. In addition, our data further indicate that KIF14 is implicated in two separate PC-related events – the formation of PC via regulation of AURA and the response of PC to Hedgehog pathway activation, with the latter being independent of AURA (Fig.8). In this regard, we have elected to change the title of our revised manuscript for "KIF14 controls primary cilium formation via Aurora A and is important for Hedgehog signaling", which we consider better suited.

Specific comments:

Manuscript would benefit from editing for grammar

The revised manuscript was edited by a native English speaker to improve the grammar and correct mistakes.

Figure 1: A missing control is knockdown of an IFT gene or kinesin-2

We have now included KIF3A siRNA condition (Fig. 1D and G) as additional control for the observed phenotypes of KIF14 RNAi.

Figure 4E. Color coding is confusing. In previous panels, KIF14 siRNA was red, CytoD was gray. Please be consistent

We are sorry for this. Red color was initially used to illustrate statistically significant changes, but we agree, that this could be quite confusing, so we have changed that. In the revised manuscript we use red color in graphs for the KIF14 siRNA condition, unless stated otherwise (experiments in Fig.5 and 7, where colors in the graphs reflect those assigned to individual conditions in the cartoons explaining the experiments).

Experiment in Figure 5 is poorly explained in the manuscript. Did authors actually measure KIF14 levels after pharmacological manipulations or is this a prediction? Supplemental videos 1-3 look very similar to my eyes. What do authors want reader to see?

Clearly, as the experiment originally shown in Fig. 5A-E raised concern from two different reviewers, we should have done better job here, and we are sorry for not being clear in presenting this particular piece of data.

In any case, we admit that there were caveats (several variables) linked to design of the experiment, and hence conclusions drawn from this data were to some extent limited (with supporting rather than decisive character). However, we believe this is mainly due to how the results were originally presented together, the data on its own are solid. With that being said, we have rearranged the data in Fig. 5 in hopefully more convincing and intuitive way and included additional experiments to support our claims. Namely, results in Fig. 5E-F (which were part of the original experiment) show that mitotic arrest is not able to phenocopy the effects of KIF14 RNAi on PC formation. In addition, data presented in Fig. 5H-N demonstrate that KIF14 RNAi leads to profound PC phenotypes even in cells arrested by serum starvation and CDK inhibition prior their transfection. In sum, we believe these data, together with data in Fig.2, provide convincing evidence that the new function of KIF14 in PC formation and signaling is related to interphase.

Authors finally show KIF14 ciliary localization in Figure 5. The manuscript would be improved if authors move this critical piece of data to the beginning of the report.

We fully agree. As we already mentioned earlier, the KIF14 localization results are now shown in Fig.2.

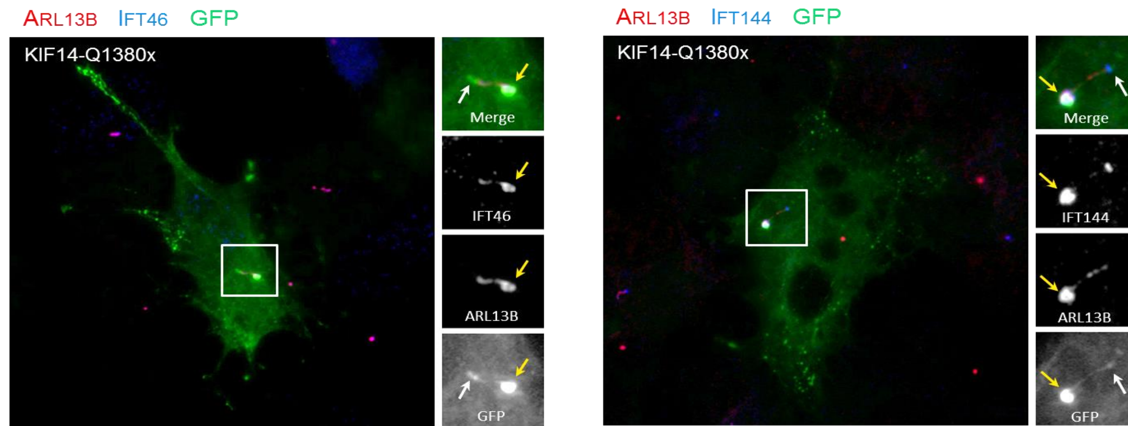
Figure 5: structure/localization studies show that the motor domain is sufficient to target KIF14 to cilia. Do these truncated GFP-KIF14 constructs have any dominant effects? In 5I, Cilia look longer and misshapen when GFP-KIF14(1-708) is expressed compared to the shorter cilia of KIF14(1-356) and (800-1648). This should be addressed.

We admit we do not fully understand the effects of KIF14 (1-708) mutant. As significant additional work would be required to solve this, we have at this point decided to remove this data and in a way 'replace' them by new data showing the localization of endogenous and transfected wt KIF14 to cilia and the effect of patient's mutations (Fig.2).

The rationale for using the missense and human disease KIF14 mutants should be better explained and more thoughtfully interpreted. How does the motor dead R364C KIF14 localize to cilia? Does this imply transport to cilia via another motor? How does the T456M mutation affect KIF14? Why would truncated mutants localize to the ciliary tip whereas KIF14(1-708) is evenly distributed along the primary cilium.

N-terminal KIF14 motor domain mutants, namely R364C and T456M, were demonstrated previously to severely impair activity of the motor domain of KIF14 (Reilly et al., 2018). In agreement with observation, we showed that they are able to localize to PC, but to a much lesser extent than KIF14 WT, demonstrated by the diminished GFP signal intensity in the ciliary tip of cells transfected with individual KIF14 constructs (Fig. 2G). The fact that both "motor dead" mutants were able to get inside the PC, albeit with reduced efficiency, can be indeed explained by activity by different motor, as suggested by this reviewer. These experiments were carried out in KIF14 WT background, so it is plausible the presence of wild type KIF14 is somewhat responsible for this observation. Alternatively, mutations in the motor domain do not 100% impair its activity, resulting in the residual levels in PC.

C-terminal KIF14 mutantions (Q1380*, Q1304*, R1189*) used in our study give rise to truncated protein moieties. This means their motor domain should be fully functional, but their ability to participate in either intermolecular or intramolecular interactions is expected to be hampered (Reilly et al., 2018; Verhey et al., 2011). In agreement with this assumption, all these mutants showed prominent accumulation in the tip of PC, which we suspect relates to their constitutively active character (see also (Reilly et al., 2018)) and in turn the inability to reload on retrograde transport complexes to be transported back to ciliary base. Noteworthy, we observed accumulation of IFT complexes in ciliary tip of cells transiently expressing any of the C-terminal KIF14 mutants (see below), reminiscent of defects in retrograde transport.



All these data demonstrate that the disease-associated KIF14 mutants examined are able to enter the PC, but their dynamics inside the PC seems to be affected, which in turn manifests as defects in their distribution in the PC. We have now included a paragraph on that in the discussion (page 15-16).

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

This is an interesting paper that presents an unexpected ciliogenesis requirement for KIF14, a motor protein generally thought of as required for cytokinesis, although in the hands of the authors, this effect is not obvious. The authors present data that clearly indicates the requirement of KIF14 in ciliogenesis and the growth of cilia. This appears to be mediated through an effect on the subdistal appendages of the basal bodies. Despite failing to see a clear involvement of KIF14 in cytokinesis, the authors do find that knockdown of the cytokinesis regulator, Citron kinase, also has a ciliogenesis phenotype.

The question is - what does KIF14 actually do in ciliogenesis. The authors are able to show its association with primary cilia and that KIF14 knockdown leads to activation of the cilia resorption pathway apparently via Aurora A. However, at the end of the paper, we are still left with an uncertainly about mechanism. For this reason, I do not believe that paper has reached the stage at which publication in JCB is appropriate although it may find a home in other cell biology journals.

As our study is the first to report novel and unexpected link between KIF14 and ciliogenesis, we believe it is understandable that it brings up many new questions, which simply cannot be answered

all at once. However, we agree with this reviewer that the question of mechanism of action of KIF14 in PC formation deserves some more probing already in this manuscript. From this perspective, we have now included additional data. To support our findings, we show that endogenous KIF14 is able to localize to PC (Fig. 2C). To extend information about mechanism, we have focused on two pertinent questions 1) Are all phenotypes of KIF14 depletion mediated by AURA? 2) How does absence of KIF14 lead to activation of AURA?

As for the former, the related data are shown Fig. 6H-M, Fig.7, Fig. 8, Fig. S4C-F, and Fig. S5A. Together, these results show AURA is responsible for the altered localization of DA components FBF-1 and SCTL1, localization of IFT-B components, and reduced PC formation and PC length in KIF14-depleted cells. To the best of our knowledge, this is the first time AURA has been implicated in the control of biogenesis of distal appendages in human cells. Intriguingly, while our data demonstrate that overexpression of AURA is sufficient to mimic all the aforementioned KIF14 RNAi defects and, in turn, its inhibition can rescue them, AURA inhibition fails to restore responsiveness of PC to Hedgehog pathway activation by SAG in KIF14-depleted cells (Fig. 8D-G). This conceptually important evidence suggests that KIF14 plays an additional, AURA-independent role in PC related to Hedgehog signaling. While the aberrant AURA activation seems to mediate most PC-related phenotypes linked to KIF14 depletion (Fig. 6-7), defects in SMO localization and induction of HH pathway target gene *Gli1* are not linked to deregulated AURA, as the "rescued PC" in KIF14 RNAi + AURA condition still fail to respond to SAG treatment (Fig.8). This strongly suggests that KIF14 function in the PC formation (via AURA) and the Hedgehog signaling (independently of AURA) are two separate phenomena.

In addition, we examined what factors mediate the activation of AURA after KIF14 siRNA. We compiled a list of proposed regulators of AURA activity, namely AJUBA, BORA, NEDD9, PIFO/Pitchfork, Trichoplein, TPX2, and Calmodulin. Our data, shown in Suppl. Fig. 5D-E, imply that AURA activation following KIF14 depletion is rather complex, as individual depletion/inhibition of tested AURA activators showed only modest rescue effects of the length of PC (Fig. S5E). Similarly, the rescue effects on the incidence of PC formation were again moderate, with the effects of NEDD9 and PIFO siRNAs reaching the level of statistical significance (Fig. S5D), but still showing only partial rescue effect, in contrast to the effect on AURA inhibition (Fig.7H-J). This suggest that either several AURA activators participate to allow efficient AURA activation in KIF14 depleted cells (and hence their co-depletion would be required to achieve a full recue of KIF14 siRNA), or AURA activity is in this case regulated by currently unknown factor(s). Clearly, additional work will be necessary to get a complete picture of how individual players participate in the AURA activation upon KIF14 depletion.

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

In this manuscript, Pejskova et al conducted loss-of-function studies on the motor protein KIF14 in RPE1 cells. In contrast to the known function of KIF14 in mitosis/cytokinesis (demonstrated previously by both in vivo and in vitro studies), the authors here found that loss of KIF14 in RPE1 cells has no/little impacts on cytokinesis/mitosis, but severely abolishes ciliogenesis instead. They showed that ciliogenesis defects are likely due to the loss of KIF14's function in interphase rather than mitosis, and that overexpressed KIF14 can localize to the ciliary compartment. Moreover, the authors demonstrated that in response to KIF14 knockdown, aurora A kinase (AURA), an enzyme known to promote cilia disassembly, is ectopically activated at the centrosome, and that inactivation of AURA can fully rescue the ciliogenesis defect, restoring both cilia growth and length in KIF14-depleted cells. Together, the authors conclude that KIF14 has a novel role in promoting ciliogenesis through regulation of the AURA kinase activity.

Major concern:

1. The function of KIF14 in mitosis, including chromosome alignment/segregation and cytokinesis, has been well demonstrated previously through both in vivo and in vitro experiments, including in Drosophila, mice, zebrafish, human patients, and several tissue culture cell lines (e.g. HeLa cells_Gruneberg el al, 2006; IMCD3 & RPE1 cells_ Reilly et al, 2018). In some of the cellular studies, ciliogenesis was specifically examined (e.g. in KIF14-/- knockout IMCD3 cells or RPE1 cells where KIF14 was depleted by siRNA), but no defect in cilia assembly was found in any case (Reilly et al, 2018). In vivo, mutation of KIF14 in mice or human was shown to cause microcephaly, and at least in human, cytokinesis failure was indeed seen in patient cells, which was believed to be the cellular defect driving microcephaly (Moawia A el al, 2017). In most cases, microcephaly is a result of stress responses that react to defects in cellular processes such as DNA replication and mitosis. Lack of ciliogenesis per se during brain development does not cause microcephaly.

The KIF14 phenotype described in the current manuscript, i.e. cilia defects but no mitotic stress, are largely inconsistent with these previous studies. I notice that the authors tried to discuss this issue in the discussion, but it is not exactly to the point, so I am not sure what to make of it, or what data to believe (although in vivo data is often more reliable). I think the authors need to experimentally reconcile these very serious discrepancies (see below).

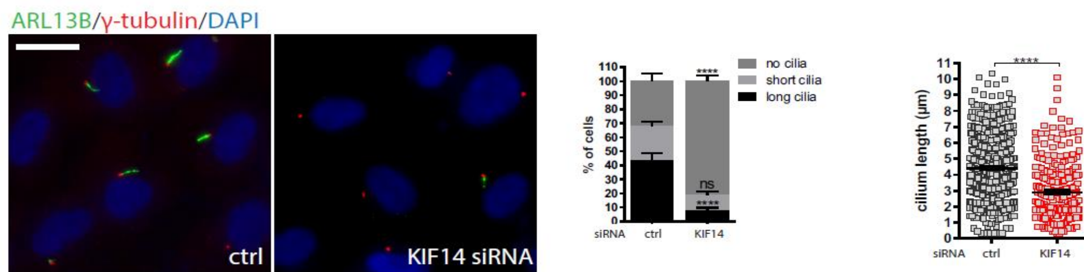
Another note: Some cells (including RPE1 cells) can overcome the defects in cytokinetic abscission, as they often migrate vigorously and can physically break the bridges. Lack of binuclear cells does not mean that cytokinesis or mitosis is perfect.

As pointed out by this reviewer, there is compelling evidence based on data from several model systems arguing KIF14/CIT does play a role in mitosis/cytokinesis. We never stated otherwise, the studies demonstrating role of KIF14 in mitosis/cytokinesis were cited in our manuscript (Bassi et al., 2013b; Carleton et al., 2006; Gruneberg et al., 2006; Moawia et al., 2017; Ohkura et al., 1997; Reilly et al., 2018; Zhu et al., 2005). However, we would like to point out that the work by Reilly and colleagues (Reilly et al., 2018), referred to by this reviewer as evidence for the role KIF14 in cytokinesis, indeed reported cytokinesis defects in IMCD3 KIF14 knockout cells, but failed to see any in zebrafish morphants. Moreover, Reilly and colleagues did not analyze cytokinesis defects in detail in RPE-1 cells that would allow strong statement on that. In any case, we argue the mitotic role of KIF14 does not exclude that KIF14 loss of function has additional consequences, which may have been missed in previous studies, in part due to more penetrant mitotic phenotypes. We believe we used rather careful wording in our manuscript to state that the function of KIF14 in PC formation does not have to be mutually exclusive with its earlier described functions. In fact, we think that we were able to detect the KIF14 depletion-related PC phenotypes exactly thanks to low penetrance of mitosis/cytokinesis defects in our models.

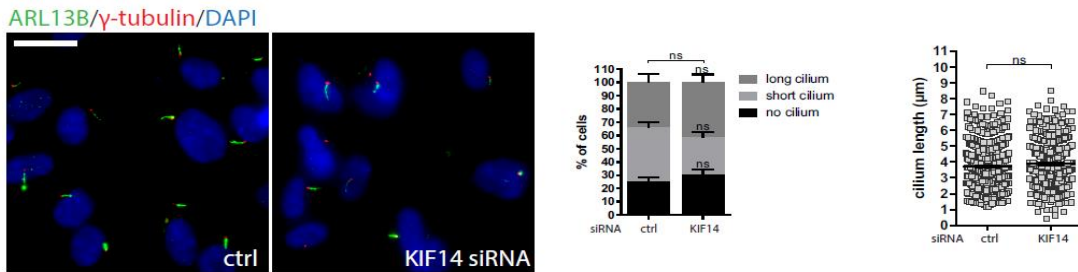
There might be several other reasons as to why depletion/absence of KIF14 was not linked to PCs defects in earlier studies: (i) KIF14 may regulate PC formation only in specific cell types. In fact, this possibility has support in earlier reports describing similar cell-type restricted requirement for efficient cytokinesis (or penetrant cytokinesis defects from loss of function) for KIF14 or CIT in *Drosophila* (Ohkura et al., 1997), in mouse development (Di Cunto et al., 2000) and in humans (Brain and kidney only (Filges et al., 2014; Reilly et al., 2018)). Moreover, as we mentioned in our manuscript (Discussion, page 15), rats with CIT loss of function mutation have been reported to have shorter PCs in a subset of neuronal cells (located in neocortex and hippocampus) (Anastas et al., 2011), again arguing for cell-context specific role. In experiments specifically requested by another reviewer, we showed that KIF14 siRNA impairs PC formation also in hESCs, hiPSCs, and IMCD3 (Fig. S1N-V), but still we cannot formally exclude KIF14 does play a context-specific role *in vivo*. (ii) The role of KIF14 in PC formation may be redundant with yet unknown factor, and in turn its deficit may

not be sufficiently penetrant in a model of constitutive knockout, due to the effect of genetic compensation (reviewed (El-Brolosy and Stainier, 2017)). In fact, different phenotypes between acute depletion and long term loss have been reported for several regulators of PC formation (Hall et al., 2013; Yadav et al., 2016). This phenomenon may in principle explain the reported lack of PC phenotype in KIF14-/- IMCD3 cells, in contrast to defective PC formation in IMCD3 following acute KIF14 depletion (Fig. S1T-V). We have included a paragraph in the Discussion on that topic.
As for the differences between this manuscript and the previous work (Reilly et al, 2018) in terms of presence/absence of PC phenotypes following KIF14 RNAi in RPE-1 cells, we would like to point out we tested one KIF14 siRNA oligo, previously used in Reilly et al, 2018 and found it to lead to comparable PC defects in RPE-1 as the other two KIF14 siRNA we originally used (Fig. S1D-F). We admit this is puzzling, we again suspect there may be some compensatory mechanism in play, given that in our protocols we analyzed PC formation 48 hours after siRNA transfection, while in the protocol used in (Reilly et al., 2018) the cells were examined 96 hours post transfection. Indeed, this argument is supported by our observations that KIF14 siRNA does not lead to notable PC defects 96 hours post transfection using protocol from "Reilly et al" (see below), but importantly the transfection with identical KIF14 siRNA every 48h leads to defective PC formation 96 hours post initial transfection ("modified Reilly et al protocol". This suggests that the penetrance of the PC phenotypes might be linked to the efficiency and/or dynamics of KIF14 depletion.

Modified Reilly et al protocol: KIF14 siRNA transfection - 48h in 10%FBS - additional KIF14 siRNA transfection - 48h in SFM - fixation



Original Reilly et al protocol: KIF14 siRNA transfection - 48h in 10%FBS - 48h in SFM - fixation



As for the comment on why we failed to see typical cytokinesis phenotypes (i.e. binucleated cells) following KIF14 depletion in RPE-1 (and human fibroblast). First, we would like to emphasize that differences in phenotypic penetrance or cell-context specific defects related to cytokinesis defects have reported for several models, including RPE-1 (McKenzie and D'Avino, 2016), neurons in mouse brain (Di Cunto et al., 2000), or zebrafish embryo (Reilly et al., 2018). We agree with the point raised by the reviewer that such defects can be masked by extensive migration of the cells and are aware that the absence of gross phenotype does not necessary mean that the process in question is without a flaw. In this regard, we would like to stress out that we did observe modest effects of KIF14/CIT depletion on cell cycle progression and mitotic timing (the increase in G2/M cells on FACS

and the increase in pH3+ cells in IF, (Fig. S3A-D, J-K, N-O), which are consistent with the reports on the mitotic role of KIF14/CIT and the reports on lower penetrance of mitotic phenotypes mentioned earlier. However, as we also discuss further, our data suggest that stress generated by prolonged mitosis (monastrol treatment followed by shake-off of mitotic cells and their release) does not lead to similar PC defects as KIF14 depletion (Fig. 5E-F).

2a. All data indicates that KIF14 is important for chromosome alignment/segregation or cytokinesis. Loss of KIF14 should therefore generate some forms/degrees of mitotic stress or insult to the cell. Even though a cytokinesis defect was not seen in this study, the authors cannot simply assume that their KIF14-depleted RPE1 cells are free of mitotic stress/insult. For example, is there a mild mitotic delay in response to KIF14 knockdown in RPE1 cells (should be)? If yes, does the mitotic delay correlate with the ciliogenesis defect seen in the coming G1 phase? Live-cell imaging with some correlated studies should work.

As we mentioned above, we believe our data are consistent with reports on the role of KIF14 or CIT in mitosis. We would like to draw your attention to Fig. S3A-B, and J-K, which present data on detection of mild phenotypes in the accumulation of G2/M cells by FACS and the percentage of pH3+ cells by IF (Fig. S3C-D, and N-O), as hypothesized by this reviewer. Importantly, this observation is consistent to that of Reilly and colleagues, who observed mild increase of mitotic cells and the decreased percentage of PC in the otic vesicle in zebrafish (Reilly et al., 2018). We would like to emphasize here that the modest effects of KIF14 depletion on G2/M cells were observed only in serum cultured cells. When the cells were serum starved, the proliferation-linked phenotypes of KIF14 siRNA were undetectable (Fig. 5A-D), which is consistent with our hypothesis that KIF14 actions towards PC formation are not caused by defective mitosis. This model of interphase-related role of KIF14 is further supported by following data: 1. Experiment presented in Fig. 5E-F implies that prolonged mitosis (and stress related to that) induced by mitotic poison monastrol, does not phenocopy KIF14 siRNA PCs defects (see also further). 2. Endogenous KIF14 localizes to PC (Fig. 2C). 3. KIF14 depletion leads to defect in PC formation even in cells arrested by serum starvation/roscovitine treatment (CDK inhibitor) prior the siRNA transfection (Fig.5H-N). Together, all these experiments argue that impaired ciliogenesis following KIF14 depletion is not a simple reflection of possible mitotic delay or effects on cell cycle. Given that our data demonstrate KIF14 siRNA leads to defective PC formation in cells that exited the cell cycle before they were actually transfected with the siRNA, we conclude that KIF14 has a novel function in PC formation in interphase, which seems independent of its role in mitosis/cytokinesis.

2b. The approach used in Figure 5 experiments is confusing; it is not clear to me how the authors can be sure about the protein level of KIF14 in each condition (mRNA level is not a direct indicator), especially when these cells are not under the same proliferation condition where we can assume all cells have the same protein turnover rate for KIF14. I have not seen this type of approach before, and I am not sure it is valid/flawless for the purpose.
Clearly, as the experiment originally shown in Fig. 5A-E raised concern from two different reviewers, we should have done better job here, and we are sorry for not being clear in presenting this particular piece of data.
In any case, we admit that there were caveats (several variables) linked to design of the experiment, and hence conclusions drawn from this data were to some extent limited (with supporting rather than decisive character). However, we believe this is mainly due to how the results were originally presented together, the data on its own are solid. With that being said, we have rearranged the data in Fig. 5 in hopefully more convincing and intuitive way and included additional experiments to support our claims. Namely, results in Fig. 5E-F (which were part of the original experiment) show that mitotic arrest is not able to phenocopy the effects of KIF14 RNAi on PC formation. In addition, data presented in Fig. 5H-N demonstrate that KIF14 RNAi leads to profound PC phenotypes even in

cells arrested by serum starvation and CDK inhibition prior their transfection. In sum, we believe these data, together with data in Fig.2, provide comprehensive evidence that the new function of KIF14 in PC formation and signaling is related to interphase.

2c. Most of mitotic stresses can lead to various degrees of p53 activation, which can globally alter the physiology of the stressed cell after mitosis. An interesting correlation that supports this notion is that IMCD3 cells are p53 compromised (SV40 transformed), and that IMCD3 can grow normal cilia in the absence of KIF14 (Reilly et al, 2018). My suggestion is that perhaps the authors can repeat the KIF14 knockdown experiment in p53-/- RPE1 cells that grow cilia, and check if cilia assembly occurs normally compared to control RNAi.

We fully agree that mitotic stress such as prolonged mitotic timing is considered as a typical upstream trigger of p53 activation (Orth et al., 2012). Indeed, we as well others have showed that the use of mitotic drugs such as nocodazole, taxol, or monastrol leads to activation of the p53 pathway which in turn triggers apoptosis, differentiation, or cell cycle arrest (Bazzi and Anderson, 2014; Orth et al., 2012; Renzova et al., 2018). However, p53-mediated stress, induced by UV irradiation, has been actually implicated as a factor triggering PC formation (Villumsen et al., 2013). As we already mentioned earlier, we did in fact introduce the factor of mitotic stress (and in turn p53 activation) into our experimental paradigm. Our data suggest that mitotic stress induced by prolonged mitosis (treatment with mitotic poison monastrol) has only modest impact on subsequent PC formation (Fig. 5C) and shows no effect on the length of PC (Fig. S3Q), in contrast to effects of KIF14 siRNA. Importantly, these experiments also show that cells that experienced monastrol-mediated mitotic arrest readily respond to KIF14 RNAi. Specifically, following the KIF14 depletion they show lower incidence of PC formation and reduced length of the formed PCs, in comparison to cells exposed to monastrol alone. This suggests that mitotic stress/p53 activation is unlikely a major mediator of the observed PC phenotypes following KIF14 siRNA. This conclusion is in line with the lack of any detectable elevation of p53 levels in KIF14 depleted cells (Fig. S3Q).

3. An alternative way to test the role of KIF14 specifically in interphase is to perform the rescue experiment specifically in G0-arrested cells, i.e. KIF14-depleted RPE1 cells are serum starved first, followed by expression of RNAi-resistant KIF14 (using lentivirus that carries the RNAi-resistant KIF14 expression construct). I would be very careful with the cell line carrying the Tet-On system, as the TRE promoter is always leaky.

We are grateful for this suggestion. Although we were not able to follow on this advice completely, due to technical limitations we encountered, we managed to obtain data demonstrating that depleting KIF14 in arrested cells (arrested by serum starvation and CDK inhibition prior siRNA transfection; Fig. S3R-S), still produced notable defects in PC formation (Fig.5H-N), supporting our original conclusions (see also our replies to previous comments).

4. The localization of the endogenous KIF14 during ciliogenesis should be examined, as over-expression can often cause artifacts or ectopic localizations, given that KIF14 is a MT binding protein and cilia are made of MT.

We are again grateful for this suggestion. We have now included data on the localization of endogenous KIF14 to PC in Fig. 2C. However, we do not agree with second part of the comment of this reviewer, implying that localization of exogenously expressed KIF14 along PC axoneme is a mere reflection of its MTs-binding capabilities and hence artificial. First, we did observe exogenous KIF14 to specifically localize along the axoneme, and not to cytoskeletal microtubules (Fig. 5F-G). Second, we tested localization of several other KIF proteins with MTs-binding ability, and only some of those, including KIF14, were able to enter the PC, while others, such as KIF2A, KIF2C, KIF24, and KIF9 did not

(data not shown). We argue that before KIF14 can bind to axonemal MTs, it has to specifically cross the transition zone with ciliary gate that otherwise prevents the entry of cytosolic proteins into the PC.

List of references:

Anastas, S.B., Mueller, D., Semple-Rowland, S.L., Breunig, J.J., and Sarkisian, M.R. (2011). Failed Cytokinesis of Neural Progenitors in Citron Kinase–Deficient Rats Leads to Multiciliated Neurons. Cereb Cortex *21*, 338–344.

Bassi, Z.I., Audusseau, M., Riparbelli, M.G., Callaini, G., and D'Avino, P.P. (2013). Citron kinase controls a molecular network required for midbody formation in cytokinesis. Proc Natl Acad Sci U S A *110*, 9782–9787.

Bazzi, H., and Anderson, K.V. (2014). Acentriolar mitosis activates a p53-dependent apoptosis pathway in the mouse embryo. PNAS 201400568.

Bernabé-Rubio, M., and A Alonso, M. (2017). Routes and machinery of primary cilium biogenesis. Cellular and Molecular Life Sciences: CMLS *74*.

Carleton, M., Mao, M., Biery, M., Warrener, P., Kim, S., Buser, C., Marshall, C.G., Fernandes, C., Annis, J., and Linsley, P.S. (2006). RNA interference-mediated silencing of mitotic kinesin KIF14 disrupts cell cycle progression and induces cytokinesis failure. Mol. Cell. Biol. *26*, 3853–3863.

Cole, D.G., Diener, D.R., Himelblau, A.L., Beech, P.L., Fuster, J.C., and Rosenbaum, J.L. (1998). Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in Caenorhabditis elegans sensory neurons. J. Cell Biol. *141*, 993–1008.

Di Cunto, F., Imarisio, S., Hirsch, E., Broccoli, V., Bulfone, A., Migheli, A., Atzori, C., Turco, E., Triolo, R., Dotto, G.P., et al. (2000). Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. Neuron *28*, 115–127.

El-Brolosy, M.A., and Stainier, D.Y.R. (2017). Genetic compensation: A phenomenon in search of mechanisms. PLOS Genetics *13*, e1006780.

Endoh-Yamagami, S., Evangelista, M., Wilson, D., Wen, X., Theunissen, J.-W., Phamluong, K., Davis, M., Scales, S.J., Solloway, M.J., de Sauvage, F.J., et al. (2009). The mammalian Cos2 homolog Kif7 plays an essential role in modulating Hh signal transduction during development. Curr. Biol. *19*, 1320–1326.

Filges, I., Nosova, E., Bruder, E., Tercanli, S., Townsend, K., Gibson, W.T., Röthlisberger, B., Heinimann, K., Hall, J.G., Gregory-Evans, C.Y., et al. (2014). Exome sequencing identifies mutations in KIF14 as a novel cause of an autosomal recessive lethal fetal ciliopathy phenotype. Clin. Genet. *86*, 220–228.

Gruneberg, U., Neef, R., Li, X., Chan, E.H.Y., Chalamalasetty, R.B., Nigg, E.A., and Barr, F.A. (2006). KIF14 and citron kinase act together to promote efficient cytokinesis. J. Cell Biol. *172*, 363–372.

Hall, E.A., Keighren, M., Ford, M.J., Davey, T., Jarman, A.P., Smith, L.B., Jackson, I.J., and Mill, P. (2013). Acute Versus Chronic Loss of Mammalian Azi1/Cep131 Results in Distinct Ciliary Phenotypes. PLOS Genetics *9*, e1003928.

He, M., Subramanian, R., Bangs, F., Omelchenko, T., Liem, K.F., Kapoor, T.M., and Anderson, K.V. (2014). The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. Nat. Cell Biol. *16*, 663–672.

Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat. Rev. Mol. Cell Biol. *10*, 682–696.

Kim, S., Lee, K., Choi, J.-H., Ringstad, N., and Dynlacht, B.D. (2015). Nek2 activation of Kif24 ensures cilium disassembly during the cell cycle. Nat Commun *6*, 8087.

Kobayashi, T., Tsang, W.Y., Li, J., Lane, W., and Dynlacht, B.D. (2011). Centriolar kinesin Kif24 interacts with CP110 to remodel microtubules and regulate ciliogenesis. Cell *145*, 914–925.

Makrythanasis, P., Maroofian, R., Stray-Pedersen, A., Musaev, D., Zaki, M.S., Mahmoud, I.G., Selim, L., Elbadawy, A., Jhangiani, S.N., Coban Akdemir, Z.H., et al. (2018). Biallelic variants in KIF14 cause intellectual disability with microcephaly. Eur. J. Hum. Genet. *26*, 330–339.

Marszalek, J.R., Ruiz-Lozano, P., Roberts, E., Chien, K.R., and Goldstein, L.S.B. (1999). Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. PNAS *96*, 5043–5048.

McKenzie, C., and D'Avino, P.P. (2016). Investigating cytokinesis failure as a strategy in cancer therapy. Oncotarget *7*, 87323–87341.

Moawia, A., Shaheen, R., Rasool, S., Waseem, S.S., Ewida, N., Budde, B., Kawalia, A., Motameny, S., Khan, K., Fatima, A., et al. (2017). Mutations of KIF14 cause primary microcephaly by impairing cytokinesis. Ann. Neurol. *82*, 562–577.

Morris, R.L., and Scholey, J.M. (1997). Heterotrimeric Kinesin-II Is Required for the Assembly of Motile 9+2 Ciliary Axonemes on Sea Urchin Embryos. J Cell Biol *138*, 1009–1022.

Ohkura, H., Torok, T., Tick, G., Hoheisel, J., Kiss, I., and Glover, D.M. (1997). Mutation of a gene for a Drosophila kinesin-like protein, Klp38B, leads to failure of cytokinesis. Journal of Cell Science *110*, 945–954.

Orth, J.D., Loewer, A., Lahav, G., and Mitchison, T.J. (2012). Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. Mol Biol Cell *23*, 567–576.

Reilly, M.L., and Benmerah, A. (2019). Ciliary kinesins beyond IFT: Cilium length, disassembly, cargo transport and signalling. Biol. Cell.

Reilly, M.L., Stokman, M.F., Magry, V., Jeanpierre, C., Alves, M., Paydar, M., Hellinga, J., Delous, M., Pouly, D., Failler, M., et al. (2018). Loss of function mutations in KIF14 cause severe microcephaly and kidney development defects in humans and zebrafish. Hum. Mol. Genet.

Renzova, T., Bohaciakova, D., Esner, M., Pospisilova, V., Barta, T., Hampl, A., and Cajanek, L. (2018). Inactivation of PLK4-STIL Module Prevents Self-Renewal and Triggers p53-Dependent Differentiation in Human Pluripotent Stem Cells. Stem Cell Reports *11*, 959–972.

Schou, K.B., Mogensen, J.B., Morthorst, S.K., Nielsen, B.S., Aleliunaite, A., Serra-Marques, A., Fürstenberg, N., Saunier, S., Bizet, A.A., Veland, I.R., et al. (2017). KIF13B establishes a CAV1-enriched microdomain at the ciliary transition zone to promote Sonic hedgehog signalling. Nat Commun *8*.

Silverman, M.A., and Leroux, M.R. (2009). Intraflagellar transport and the generation of dynamic, structurally and functionally diverse cilia. Trends Cell Biol. *19*, 306–316.

Verhey, K.J., Kaul, N., and Soppina, V. (2011). Kinesin assembly and movement in cells. Annu Rev Biophys *40*, 267–288.

Villumsen, B.H., Danielsen, J.R., Povlsen, L., Sylvestersen, K.B., Merdes, A., Beli, P., Yang, Y.-G., Choudhary, C., Nielsen, M.L., Mailand, N., et al. (2013). A new cellular stress response that triggers centriolar satellite reorganization and ciliogenesis. EMBO J *32*, 3029–3040.

Yadav, S.P., Sharma, N.K., Liu, C., Dong, L., Li, T., and Swaroop, A. (2016). Centrosomal protein CP110 controls maturation of the mother centriole during cilia biogenesis. Development *143*, 1491–1501.

Zhu, C., Zhao, J., Bibikova, M., Leverson, J.D., Bossy-Wetzel, E., Fan, J.-B., Abraham, R.T., and Jiang, W. (2005). Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference. Mol. Biol. Cell *16*, 3187–3199.

March 11, 2020

RE: JCB Manuscript #201904107R-A

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Dear Dr. Cajanek:

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

Authors did a thorough job of addressing all of my previous concerns with additional experiments and changes to the text. The reorganization of the manuscript, new data on KIF14 knockdown in multiple cell lines, and localization of endogenous KIF14 in the cilium all make for a convincing story. In my original review, I forgot to include this comment. Authors introduce unnecessary abbreviations: PC for primary cilia/cilium is unnecessary; DA for distal appendages.