Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens

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Original submission

First decision letter

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MS TITLE: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens

AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees' opinions on suitability for publication in Development are mixed. However, as all referees make constructive criticisms and suggestions for improvements, I would be happy to receive a revised version of the manuscript if you are able to tackle the criticisms and revise the manuscript along the lines suggested. Please also note that Development will normally permit only one round of major revision.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

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Reviewer 1

Advance summary and potential significance to field

In this paper Yumei Hao and colleagues make use of the zebrafish lens and retina development to address in vivo the coordination between the small GTPase Rab11, adherens junction (AJ) remodeling and polarity proteins. This is an important issue since tissue morphogenesis require an important interconnection between membrane trafficking and cell polarity but the mechanisms involved are still largely unknown. The authors report that upon epidermal to epithelial transition in zebrafish lens, MPP5a a key element of the Crumbs polarity complex interacts with the rab11 GTPase to orientate the membrane traffic of crumbs complex and Ajs components and therefore building apical polarity.

Comments for the author

This work is associated with many interesting observations but at the same time, I feel that a number of conclusions are not fully supported by the results presented. In addition, a number of interesting results are not presented in a very understandable way.

Detailed Comments

AJs remodeling during lens state transition Electron microscopy images are interesting but the conclusions made from some images are difficult to follow especially for the panels 1i to 1k. The scheme in 1M is quite difficult to understand, the color code could be better linked with the TEM pictures.

AJs components in nok mutants and interplay between Nok and Rab11 The results clearly show that in nok mutants, AJs components fail to accumulate at the apical domain in lens epithelia. They further show that in nok mutant, Rab11 loses its apical localization, although it would have been nice to have an idea of the number of samples analyzed. They then show that in double mutant rab11a/rab11ba Crb2A is absent.
How Crb2a is located in nok mutant ?
I have difficulties of interpretation for the experiments with the dominant negative version of Rab11 S25N (fig. 3K), for me Nok is still apically located and the basal signal highlighted by the broken arrows could well correspond to background, again no there is no mention to the number of samples analysed

MPP5a, PAR proteins and Rab11
The authors have investigated the potential interactions between rab11, MPP5a and Par proteins especially those involved in apical identity. An important concern is that co-immunoprecipation experiments were performed in HEK293T human cells after transfection of fish cDNAs. It is difficult to draw conclusions on results in such cases.
I agree that the interaction between rab11 and MMP5 is supported by the results with rab11Q70L, rab11 S25N and the subsequent domain mapping analysis. But the negative results do not allow to conclude on a lack of interactions with Par proteins. The results would be stronger if they had been made from zebrafish lens extract with co-immunoprecipation between Rab11, Nok and apical Par proteins.
The effect of Crb or Par proteins P on AJ distribution by following b-cat is difficult to interpret from the data. The difference between fig.4F versus fig.4G and fig.4H versus fig.4I are not striking and no details for the quantification are provided.
I have the same difficulties with the analysis of Nok localization with the secretory pathway especially in human cells.
I am also surprised that the authors follow only the track of Rab11 in the secretory pathway while Rab11 involvement of endocytic pathway recycling is also largely established and could account for AJ remodeling.

Par complex organizes cytoskeleton..
The co-immunoprecipation results obtained in human cell experiments are not demonstrative to allow a conclusion about a distinct mechanism concerning Par complex for AJs remodelling. I found hardly convincing the results indicating that Rab11 localization was associated with Crb complex

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but not aPKC. In fig.S6, aPKC remains apically localized in rab11 mutant lens, the epithelium in the image is much less disorganized compare to the fig.3F.
From the data, it is very difficult to conclude that F-actin is closely associated with aPKC than with the Crb complex.

Reviewer 2

Advance summary and potential significance to field

I have reviewed a manuscript submitted by Hao et al., which is entitle “Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens. The authors focus on AJ formation in the zebrafish lens. They found that an apical regulator Crumbs2a and a component of AJs, beta-catenin, fail to be localized in the apical junctional domain between lens epithelial cells in zebrafish rab11a and rab11ba double mutants. They also showed that apical domain localization of Nok is compromised in lens epithelial cells overexpressing dominant-negative rab11a. Furthermore, rab11 protein is normally localized in the apical domain of lens epithelium in zebrafish but not in nok mutants, suggesting that apical targeting of Nok and rab11 is reciprocally dependent each other. Consistently, rab11 physically binds Nok in human in vitro cultures and both positive signals are localized in Golgi, suggesting that rab11-mediatded exocytosis underlies these phenotypes. Lastly, the authors investigated whether a component of Par complex, aPKC, is involved in this process and found that apical localization of aPKC is lost in nok mutants but less depend on rab11, suggesting that some independent mechanism of aPKC and nok/rab11 in AJ formation. Almost all the data are very interesting and the authors succeed to figure out the important role of rab11 in AJ formation in zebrafish lens epithelium.

Comments for the author

Although most of the data are valid, one of weak points is that the authors did not provide enough data to confirm that rab11 functions along exocytosis but not through endocytosis. Thus, I suggest the authors to examine whether similar phenotypes are not observed in zebrafish rab5 knockdown using overexpression of dominant-negative rab5 in lens epithelial cells, or in zebrafish lenses treated with a dynamin inhibitor such as Dynasore. Alternatively, the authors will confirm that lens phenotypes in rab11 knockdown zebrafish are not changed by rab5 knockdown or Dynasore treatment. Furthermore, although the data are valid, there are several immature points in manuscript and figure preparation. Specific comments are provided below, and I hope that these my comments are useful for the authors to improve the manuscript.

(1) Page and line number should be assigned in the manuscript for reviewers to make comments easy.
(2) Page 4, line 3 and the first page of the discussion: The term “Drosophila” should be italic.
(3) Fig. 1A-D: Was these images obtained by whole mount labeling of anti-E-cadherin antibody? Please cite references which provide the information on this antibody. Furthermore, the authors did not mention the orientation of lenses in figure legends. Since the retina is located in the left side, so anterior direction is right side. Please indicate the orientation of the lens in figure legends.
(4) There is no description of EM data shown in Figure 1E-G in the results section. Every figure panels should be cited in the text.
(5) Page 4, line 4: Abbreviation of “WT” should be shown as “wild type (WT)” at the first place.
(6) Fig. 1K, 1M was not cited in the main text of results section.
(7) Contrast of Fig. 1E and 1H is not good. Please improve it.
(8) Orientation of Fig. 1G should be consistently assigned with box 2 of Fig. 1E.
(9) Legend of Fig. 1. (E-G): The sentence “The black boxed region of I shows the apical zone between two epithelia” may be mistaken to be inserted into legend of (E-G), but should be moved into the right place of legend (H-J).
(10) Page 4, line 17: The term “dissolved” may be changed into “disassembled”.
(11) Fig. 1I: It is difficult to see the outline of lens epithelial cells and lens fiber cells. Please provide a schematic drawing. Fig. 1J orientation should be consistent with box 2 of Fig. 1H.

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(12) Figure panel configuration is not good in Fig. 1. EM image panels at the same stage ((E, F, G) at 24 hpf and (H, I, J, K) at 36 hpf) should be assembled together, for example, aligned in the same horizontal row.
Furthermore, panel size and outline also should be consistently organized.
(13) Scale bars should be shown in Fig. 1E and 1H.
(14) Orientation of lens is obscure in Fig. 2A. Fig. 2A seems to be obtained from the anterior view (lateral view of the eye), although Fig. 2B seems to be obtained from the ventral view of embryo. Please make sure the orientation of lens consistent. Furthermore, there is Nok signal in central part of the lens at 28 hpf (Fig. 2B). Is this a real signal or noise? Please carefully set the signal threshold.
(15) Fig. 2F: The increase in number of apical AJs between epithelia-fiber cells in nok mutants may be due to invasion of epithelia into inner lens. This should be mentioned in the text. How about the AJ formation between ingressed (Zl1-netative) lens epithelial cells and Zl1-positive lens fiber in nok mutants?
(16) Please mention results on the length of AJs in nok mutants in the main text by citing Fig. 2G. It may be better to merge Fig. 1L and 2G, because only length of AJ_EE (Fig. 2G) is shorter in nok mutants than in wild type (Fig. 1L).
(17) There is no citation on Fig. S3 panels (A, B, C, G, H) in the main text.
(18) Fig. S3 (FGH): Please explain why lens fiber cell number is higher in nok mutants than in wild type at 36 hpf in the main text. If the authors count nuclear number of fiber cells, denuleation (degradation of lens fiber cells) may be compromised and slower in nok mutants.
(19) Fig. 3A-F: Please cite literatures that used the rab11 antibody in zebrafish. There are Rab11 antibody signals in lens of rab11a and rab11ba mutants, and also double mutants, so I wonder which rab11 subtypes are recognized by this antibody or whether the epitope site of this antibody is located in the N-terminal region of premature stop of the rab11a and rab11ba mutants, which the authors generated by Crispr/CAS9. Please provide the more detailed information on this antibody.
(20) Fig. 3G-K: In accordance with materials and methods, this series of experiments seemed to be done using transient expression of GFP-tagged rab11 by injection of DNA construct. Please mention it in the main text. In addition, there is no description about how Rab11a Q70L-eGFP expression was introduced in materials and methods. Please revise it.
(21) Fig. 3K: The authors mentioned that Rab11a S25N severely impeded the apical localization of Nok;
however, actual phenotypes were weak. Please conduct statistical analysis and show its histogram.
(22) Fig. 3: Please show labeling of Nok antibody in rab11a/rab11ba mutants, whose signal is expected to be absent.
(23) Fig. 3 panel configuration: vertical outlines of panels (A, A', D, D') and panels (G, G', H, H') should be aligned.
(24) Fig. 4K and 4L: Overlapping of rab11, Nok, and GM130, a Golgi marker or PDIA3, an ER marker was not clear. Please show higher magnification images. Statistical analysis is also required to show the percentage of triple positive foci in rab11a-positive foci, Nok-positive foci or GM130-positive foci. It is ideal and practically possible to conduct the same experiments using zebrafish lens epithelium, rather than human in vitro culture.
(25) Fig. 4K: In contrast to nok mutants, number of plaques of AJ_EF is not different between wild-type and rab11 single mutants. This may be due to normal monolayer of lens epithelium in rab11 double mutants.
However, number of plaques of AJ_EF is reduced in rab11 double mutants. Please explain why.
(26) Fig. 6: The authors conclude that Par complex does not recruit rab11. Please indicate rab11a expression in aPKC mutants. It is interesting to investigate whether apical localization of rab11a is independent of aPKC.
(27) Fig. 6G and 6H: It is better to show the data of lens epithelium rather than surface epidermis. Is a similar tendency of cytoskeleton organization observed in Fig. 6F?
(28) Fig. 6 panel configuration should be improved. Location of panels (B-B”) is not good. Panels A-C should be vertically aligned together. Interval space between panels E/F, G, H can be smaller, then panel J will be moved into the right side.
(29) Legend of Fig. S1: Scale bar 20 um -> 20 micro-m (font change)
(30) Fig. S1C: Authors mentioned that arrowheads indicate non-specific signals. What tissues does the antibody non-specifically bind in the basal region of wild-type retina (blood vessels?)? Furthermore, are signals in the outer neural retina of the nok mutants non-specific? Please replace with better images without such noise signals.

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(31) Fig. S1F: Focus is not good and blur in these images. I suggest the authors to take image using a compound microscope, but do not enlarge images obtained by a dissecting microscope.

Reviewer 3

Advance summary and potential significance to field

In this paper, Hao and colleagues analysed the interaction between the Crumbs-MPP5a-PATJ complex (Crb complex) and the small GTPase Rab11 during the establishment of apical cell polarity in the developing lens and retina of the zebra fish. Through this interaction, the vesicle exocytosis is biased towards the apical cell domain. Interfering with either with the function of the Crb complex or the function of Rab11 results in ectopic localisation of Crb and adherens junction (AJ) components.
The authors nicely showed in vivo that the AJs are progressively established during lens development. This gradual formation of proper AJ depends on the gradual apical localisation of the Crb complex.
Hao et al. produced a great amount of work in generating nok and rab11 mutant lines using the CRISPR/Cas9 technique. They also did some live imaging of lens development in these mutant contexts.
In addition, they performed a detail analysis of AJ formation in various genetic contexts by transmission electron microscopy. However, they do not take the best advantage of this analysis, as the panels are far too small and not well organised within a figure. For example in Figure 1, it is very hard to spot the two squares 1 and 2 in panel E. These two areas are enlarged in panels F and G respectively. It would be of great help to have the three panels on the same line rather than to browse throughout the figure. The same stands true for panels H-K. Panel layout is also very confusing in Figure 4.

Comments for the author

In this paper, Hao and colleagues analysed the interaction between the Crumbs-MPP5a-PATJ complex (Crb complex) and the small GTPase Rab11 during the establishment of apical cell polarity in the developing lens and retina of the zebra fish. Through this interaction, the vesicle exocytosis is biased towards the apical cell domain. Interfering with either with the function of the Crb complex or the function of Rab11 results in ectopic localisation of Crb and adherens junction (AJ) components.
The authors nicely showed *in vivo* that the AJs are progressively established during lens development. This gradual formation of proper AJ depends on the gradual apical localisation of the Crb complex. Hao et al. produced a great amount of work in generating *nok* and *rab11* mutant lines using the CRISPR/Cas9 technique. They also did some live imaging of lens development in these mutant contexts.
In addition, they performed a detail analysis of AJ formation in various genetic contexts by transmission electron microscopy. However, they do not take the best advantage of this analysis, as the panels are far too small and not well organised within a figure. For example in Figure 1, it is very hard to spot the two squares 1 and 2 in panel E. These two areas are enlarged in panels F and G respectively. It would be of great help to have the three panels on the same line rather than to browse throughout the figure. The same stands true for panels H-K. Panel layout is also very confusing in Figure 4.

In spite of this, it is already known in many other systems that Rab11 distribution is apically bias to fully support secretion of AJ components. In addition, I don't think that authors reach their conclusions with the data they provided.
1- From Figure 3, they concluded for a reciprocal dependency of Rab11 and the Crb complex. They used a Rab11a-eGFP reporter line to follow Rab11 distribution in a *nok* mutant context. But this reporter line expression profile (panel G') does not resemble the endogenous Rab11 distribution.
2- The authors used HEK293T cells to perform co-immunoprecipitation and co-localisation assays. These cells are human cells derived from human kidney, transfected by a variety of proteins of zebra fish or human origin. I find it very confusing to ascertain with protein interacts with another one independently of the cell context. In addition, Hao et al concluded

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that the Crb complex interacts with Rab11 in the Golgi apparatus. However, they drew their conclusion from cells that do not seem to have form AJs.

3- Finally, authors jump on the relationship between the Par complex and the actin cytoskeleton. They showed that the Par complex enrichment is independent of the Crb/Rab11 complex localisation. They controlled that the Par complex may control the orientation of the actin cables, as actin fibres change orientation in a PKC mutant background. At the resolution displayed on the figure, this is quite impossible to conclude anything about actin fibres orientation and the transmission electron microscopy is not helpful. In addition, I don't think the authors can reach such strong conclusion, as it can be an indirect consequence of affecting another cytoskeleton. It would be very helpful is Hao et al. could directly disturbed the actin cytoskeleton and assessed AJs remodelling.

4- Final minor point, the figures are too small, close up will be very appreciated. In addition the schematic representation of AJ distribution is far too small. It is nearly impossible to spot the legend on the illustration. In addition, the confocal images display the developing lens on the right hand-side while the drawing shows it on the top. Why not adopting the same position?

In conclusion, I don't think this work raises the novelty high enough to fully support publication in Development and I recommend publication in a more specialised journal.

First revision

Author response to reviewers' comments

Dear Editors and reviewers,
We thank the editors and reviewers for their efforts in evaluating our manuscript and their constructive comments. We have performed new experiments to address the reviewers' concerns. In particular, we provide additional evidences, (1), to prove the interaction between Rab11 and MPP5a (Nok in zebrafish) by endogenous co-IPs using zebrafish eye and MDCK cells extracts; (2), to prove endocytosis did not play a significant role during polarity establishment of lens epithelia by over-expressing Rab5 S36N (dominant negative form of Rab5. The over-expression of Rab5 S36N did not affect the apical localization of Nok in lens epithelia; (3), to prove the over-expression Rab11a S25N dose-dependently disrupted the proper localization of Nok in lens epithelia by mRNA injection; (4), to prove the association between Crb2a and Rab11, and between aPKC and actin. These new data and revised data are shown in Fig. 1G, 1H, 2G, 3D-3F, 3I-3L, 3O, 3P, 4D-4J, 4L-4N, 5G, 6H-6J, S1, S3C, S3F, and S4.
We have also re-edited the figure configuration and added the required information (such as references for antibodies) according to the reviewers' comments. Accordingly, we have revised the text (marked with blue color in the manuscript) with necessary corrections and clearer description. We also renamed AJ_EE, AJ_EF, BJ_EE, LJ_EE as AJs_EE, AJs_EF, BJs_EE, LJs_EE. We believe that all concerns/questions raised by the reviewers have been addressed, and the revised manuscript is significantly improved. Below (marked with blue color) are our point-by-point responses to the reviewers' comments:

Reviewer 1 Advance Summary and Potential Significance to Field...
In this paper Yumei Hao and colleagues make use of the zebrafish lens and retina development to address in vivo the coordination between the small GTPase Rab11, adherens junction (AJ) remodeling and polarity proteins. This is an important issue since tissue morphogenesis require an important interconnection between membrane trafficking and cell polarity but the mechanisms involved are still largely unknown. The authors report that upon epidermal to epithelial transition in zebrafish lens, MPP5a a key element of the Crumbs polarity complex interacts with the rab11 GTPase to orientate the membrane traffic of crumbs complex and Ajs components and therefore building apical polarity.

Reviewer 1 Comments for the Author...
This work is associated with many interesting observations but at the same time, I feel that a number

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of conclusions are not fully supported by the results presented. In addition, a number of interesting results are not presented in a very understandable way.
Thanks for the reviewer's comments and suggestions. Please see below for our point-to-point responses.

Detailed Comments

AJs remodeling during lens state transition
Electron microscopy images are interesting but the conclusions made from some images are difficult to follow especially for the panels 1i to 1k. The scheme in 1M is quite difficult to understand, the color code could be better linked with the TEM pictures.
Thanks for the reviewer's comments. We have accordingly (together with reviewer 2's comments) re-edited the Figure 1. Specifically, we replaced the lower resolution TEM images (Fig. 1E and 1H in the previous version) by schematic drawings and moved to supplemental Fig. S1. We also revised the scheme (Fig. 1M in the previous version) to better show the color code. We also replaced Fig1G and 1H (Fig. 1I and 1J in the previous version), and colored the cells in lens TEM images to clearly show the cell outlines. Please also see reviewer 2's comments 7 and 12.

AJs components in nok mutants and interplay between Nok and Rab11
The results clearly show that in nok mutants, AJs components fail to accumulate at the apical domain in lens epithelia. They further show that in nok mutant, Rab11 loses its apical localization, although it would have been nice to have an idea of the number of samples analyzed. They then show that in double mutant rab11a/rab11ba Crb2A is absent.
Thanks for the reviewer's comments. In this study, for all immunohistochemistry experiments, we repeated each immunohistochemistry experiment three or more times, and 10 or more embryos were analyzed for each experiment. In another word, at least 30 embryos were examined for all immunohistochemistry experiments. For all TEM experiments, we examined at least 5 embryos for each experiment. For quantifications, the exact number (>50 cells in 5 retina from 5 embryos for TEM images; ≥10 retinas for IHC images) was shown in each figure legends and Methods/Statistics sections. Most of these information was mentioned in Methods/Quantifications and Statistics section. Now we revised the Methods/ Immunohistochemistry and antibodies, and Quantifications sections to better show the information.

How Crb2a is located in nok mutant?
Thanks for the reviewer's comments. The Nok localization in crb2a^{m289} mutants and the Crb2a localization in nok^{ZJUKO203} mutants were shown in Fig. S2C and S2D (Fig. S1C and S2D in the previous version), respectively.

I have difficulties of interpretation for the experiments with the dominant negative version of Rab11 S25N (fig. 3K), for me Nok is still apically located and the basal signal highlighted by the broken arrows could well correspond to background, again no there is no mention to the number of samples analysed.
Thanks for the reviewer's comments. The negatively regulation of Rab11a S25N on Nok localization is dose-dependent. We now provided new data for the higher level over-expression of Rab11a S25N (Fig. 3O, by plasmid injection), provided new data by Rab11a S25N-2A-eGFP mRNA injection (Fig. 3K and 3L), and re-edited the control images (Fig. 3M, over-expression of WT Rab11a). Comparing to controls (WT zebrafish in Fig.2C, and over-expression of WT Rab11a in Fig. 3K and M), we can clearly see the significant differences: sporadic expression of Rab11a S25N at lower level induced the lateral and basal enrichment of Nok in lens epithelia (Fig. 3N). More importantly, with higher level of Rab11a S25N, the apical signal of Nok was almost completely lost in lens epithelia (Fig. 3L and 3O). Together with the results from loss-of-function shown in Fig. 3G-3J), we can make a conclusion that Rab11 regulates the apical localization of Nok in lens epithelia.

MPP5a, PAR proteins and Rab11
The authors have investigated the potential interactions between rab11, MPP5a and Par proteins especially those involved in apical identity. An important concern is that co-immunoprecipitation experiments were performed in HEK293T human cells after transfection of fish cDNAs. It is difficult to draw conclusions on results in such cases. I agree that the interaction between rab11 and MMP5 is supported by the results with rab11Q70L, rab11 S25N and the subsequent domain mapping analysis. But the negative results do not allow to conclude on a lack of interactions with Par proteins. The

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results would be stronger if they had been made from zebrafish lens extract with co-immunoprecipitation between Rab11, Nok and apical Par proteins. The effect of Crb or Par proteins P on AJ distribution by following b-cat is difficult to interpret from the data. The difference between fig.4F versus fig.4G and fig.4H versus fig.4I are not striking and no details for the quantification are provided.

Thanks for the reviewer's comments. Previously we performed endogenous co-IP for the interaction of Rab11 with Pals1 (Nok homolog in mammals) in MDCK cells (Fig. 4D in previous version), a classic epithelial cell line widely used in the cellular polarity and adhesion field (Simmons, 1982; Chavrier et al., 1990; Hurd et al., 2003; Li et al., 2014; Mrozowska and Fukuda, 2016). Now we performed new endogenous co-IP experiments to examine the interaction of Rab11 with all tested proteins including Par3, Par5, Par6, aPKC and Pals1 in MDCK cells (Fig. 4D). Furthermore, we also performed the endogenous co-IP experiment using zebrafish eye extract to examine the interaction of Rab11 with Nok (Fig. 4E). For other zebrafish proteins including Par3, Par5, Par6 and aPKC, specific antibodies which could be used for zebrafish endogenous co-IP is not available. These results showed that Nok/Pals1 was the only proteins which could interaction with Rab11.

*For the β-catenin fluorescent intensity, we have re-edited Fig. 4 and marked the positive and control cells. In these cell culture studies, the best control is the neighbor cells which are transfection negative. Thus we compared the membrane associated β-catenin intensity in the transfection positive cells (marked by *) with that in the transfection negative neighbor cells (marked by #). As shown in figures, the expression of eGFP did not affect the expression and localization of β-catenin comparing with the neighbor control cells (Fig. 4F). The expression of Crb2a and Nok were highly enriched β-catenin to the cell membrane (Fig. 4G). The expression of aPKC and Pard6 promoted the level of β-catenin in cytoplasmic, but not significantly induced its membrane associated distribution (Fig. 4H). The membrane associated β-catenin intensity in both Crb2a and Rab11a positive cells were much higher than that in the neighbor control cells (Fig. 4I). However, the membrane associated β-catenin intensity in both Crb2a and Rab11a S25N positive cells was comparable with the neighbor control cells (Fig. 4J). We added the quantification information in Methods, and accordingly revised the text.*

I have the same difficulties with the analysis of Nok localization with the secretory pathway especially in human cells.
I am also surprised that the authors follow only the track of Rab11 in the secretory pathway while Rab11 involvement of endocytic pathway recycling is also largely established and could account for AJ remodeling.

Thanks for the reviewer's comments. Sorry for the misleading writing, we performed the experiments shown in Fig. 4L-4P in MDCK cells, a classic epithelial cell line widely used in the cellular polarity and adhesion field (Simmons, 1982; Chavrier et al., 2990; Hurd et al., 2003; Li et al., 2014; Mrozowska and Fukuda, 2016). We tried the cytoplasmic co-localization experiments (Rab11/Nok/Golgi) in zebrafish lens tissue. However, the endogenous Rab11 and Nok proteins could not be detected in cytoplasm, probably due to the quick exocytosis of nascent proteins after translation in living zebrafish. Similar with the endogenous proteins, the over-expression of Nok proteins could not be detected in cytoplasm.

During lens development, the lens cells go through four cellular states, starting from epidermal state, then epithelial state, followed by mesenchymal state, and then the final state lens fiber cells (Greiling et al., 2009). The apical complex Crb/Nok is only present in lens epithelial cells (Fig. 2A-2C and S2). In another word, the apical enrichment of nascent Nok proteins may be mediated by vesicle exocytosis, but not endocytosis during epidermal-to-epithelial transition. Whereas, the loss of Nok proteins in mesenchymal cells may be mediated by endocytosis during epithelial-to-mesenchymal transition. In this study, we focused on the establishment of apical polarity and zonula adherens during epidermal-to-epithelial transition (from 28 hpf to 36 hpf). Thus we previously focused on the Rab11 mediated vesicle exocytosis pathway.

Rab11 plays functions in both exocytosis and endocytosis (Ullrich et al., 1996; Chen et al., 1998; Woichansky et al., 2016). Rab5 plays a key role in endocytosis (Chavrier et al., 1990; Gorvel et al., 1991; Barbieri et al., 1996). Now we performed new experiments to prove that the dysfunction of Rab5 (by sporadic over-expression of Rab5 S36N in WT zebrafish, a dominant negative form of Rab5) did not affect the apical localization of Nok in lens epithelia (Fig. 3P), suggesting endocytosis is not involved in the Nok localization in lens epithelia during polarity establishment. Interestingly, we

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indeed observed Nok proteins was ectopically present in lens fiber cells in zebrafish lens over-expressed with either Rab5 S36N or Rab11a S25N (Fig. 3K-3P), suggesting an important role of endocytosis on breaking down the apical polarity in lens mesenchymal/fiber cells during epithelial-to-mesenchymal and fiber cells transition. Please also see the reviewer 2's comments. We have accordingly revised the text.

We also tried to analyze the function of Rab5 S36N on zonula adherens formation by TEM. Considering the sporadic positive cells cannot be detected by TEM, we injected eGFP-2A-Rab5 S36N mRNA into zebrafish embryos, an experiment similar with the over-expression of Rab11a S25N shown in Fig. 3K, 3L, 5E, 5F, and 5J. The mRNA over-expression of Rab11a S25N did not significantly block the general development of zebrafish embryos. However, different from the injection of Rab11a S25N, the mRNA over-expression of Rab5 S36N blocked the embryo development at gastrulation stage.

In this study, we focused on the novel Nok/Rab11 complex and its function in the apical polarity and zonula adherens formation in epithelia. However, we agree that how endocytosis/exocytosis pathways synergistically regulate the apical AJs remodeling (including apical zonula adherens between lens epithelia-epithelia and punctum adherens between epithelia-fiber cells) in vertebrate is a very interesting question for future studies. We revised the discussion about the function of endocytosis on apical polarity and zonula adhesions formation.

Par complex organizes cytoskeleton..
The co-immunoprecipation results obtained in human cell experiments are not demonstrative to allow a conclusion about a distinct mechanism concerning Par complex for AJs remodelling. I found hardly convincing the results indicating that Rab11 localization was associated with Crb complex but not aPKC. In fig.S6, aPKC remains apically localized in rab11 mutant lens, the epithelium in the image is much less disorganized compare to the fig.3F.
From the data, it is very difficult to conclude that F-actin is closely associated with aPKC than with the Crb complex.
Thanks for the reviewer's comments. Now we provided the endogenous co-IPs data to support the interaction between Nok/Pals1 and Rab11 (Fig. 4D and 4E).

The image (Fig. S6D in previous version) are a little bit out of the imaging focus (the imaging focus was on the retina, but not the lens). We replaced new images for Fig. 3I and Fig. 6J (Fig. 3F and Fig. S6 in the previous version) and added the actin staining in Rab11a/ba double knockouts (Fig. 6J).

In rab11a/rab11ba double KO mutants, immunostaining clearly showed that Rab11, Crb2a and Nok were all lost in lens epithelia, however, both aPKC and actin were nicely enriched at the apical regions (Fig. 3I, 3J, and 6J). In aPKCλ^{m567} mutants, the immunostaining signal of aPKC was lost, actin also lost its apical localization; however, both Rab11 and Crb2a signals were co-localized at the apical regions in some epithelia (Fig. 6H and 6I). In nok mutants, the immunostaining signals of both Rab11 and Crb2a were lost in all lens epithelia, while both aPKC and actin were enriched at lens lateral epithelia (Fig. 3C, 6K, and 6L). Taken together, we believe that all these data support the conclusion that the localization of Rab11 is associated Crb2a with but not actin; and the localization of actin is associated with aPKC, but not Crb2a.

We have accordingly revised the text.

Reviewer 2 Advance Summary and Potential Significance to Field...

I have reviewed a manuscript submitted by Hao et al., which is entitle "Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens. The authors focus on AJ formation in the zebrafish lens. They found that an apical regulator Crumbs2a and a component of AJs, beta-catenin, fail to be localized in the apical junctional domain between lens epithelial cells in zebrafish rab11a and rab11ba double mutants. They also showed that apical domain localization of Nok is compromised in lens epithelial cells overexpressing dominant-negative rab11a. Furthermore, rab11 protein is normally localized in the apical domain of lens epithelium in zebrafish but not in nok mutants, suggesting that apical targeting of Nok and rab11 is reciprocally dependent each other. Consistently, rab11 physically binds Nok in human in vitro cultures, and both positive

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signals are localized in Golgi, suggesting that rab11-mediatded exocytosis underlies these phenotypes. Lastly, the authors investigated whether a component of Par complex, aPKC, is involved in this process and found that apical localization of aPKC is lost in nok mutants but less depend on rab11, suggesting that some independent mechanism of aPKC and nok/rab11 in AJ formation. Almost all the data are very interesting and the authors succeed to figure out the important role of rab11 in AJ formation in zebrafish lens epithelium.

Reviewer 2 Comments for the Author...

Although most of the data are valid, one of weak points is that the authors did not provide enough data to confirm that rab11 functions along exocytosis but not through endocytosis. Thus, I suggest the authors to examine whether similar phenotypes are not observed in zebrafish rab5 knockdown using overexpression of dominant-negative rab5 in lens epithelial cells, or in zebrafish lenses treated with a dynamin inhibitor such as Dynasore. Alternatively, the authors will confirm that lens phenotypes in rab11 knockdown zebrafish are not changed by rab5 knockdown or Dynasore treatment. Furthermore, although the data are valid, there are several immature points in manuscript and figure preparation. Specific comments are provided below, and I hope that these my comments are useful for the authors to improve the manuscript.

Thanks for the reviewer's comments and suggestions. Now we performed new experiments to prove that the dysfunction of Rab5 (by sporadic over-expression of Rab5 S36N in WT zebrafish, a dominant negative form of Rab5) did not affect the apical localization of Nok in lens epithelia (Fig. 3P), suggesting endocytosis is not involved in the Nok localization in lens epithelia during polarity establishment. Interestingly, we indeed observed Nok proteins was ectopically present in lens fiber cells in zebrafish lens over-expressed with either Rab5 S36N or Rab11a S25N (Fig. 3K-3P), suggesting an important role of endocytosis on breaking down the apical polarity in lens mesenchymal/fiber cells during epithelial-to-mesenchymal and fiber cells transition. Please also see the reviewer 2's comments. We have accordingly revised the text.

We also tried to analyze the function of Rab5 S36N on zonula adherens formation by TEM. Considering the sporadic positive cells cannot be detected by TEM, we injected eGFP-2A-Rab5 S36N mRNA into zebrafish embryos, an experiment similar with the over-expression of Rab11a S25N shown in Fig. 3K, 3L, 5E, 5F, and 5J. The mRNA over-expression of Rab11a S25N did not significantly block the general development of zebrafish embryos. However, different from the injection of Rab11a S25N, the mRNA over-expression of Rab5 S36N blocked the embryo development at gastrulation stage. In addition, Dynasore treatment did not work well in zebrafish. We revised the discussion about the function of endocytosis on apical polarity and zonula adhesion formation.

(1) Page and line number should be assigned in the manuscript for reviewers to make comments easy.

Thanks and we have accordingly revised the text.

(2) Page 4, line 3 and the first page of the discussion: The term "Drosophila" should be italic.

Thanks and we have accordingly revised the text.

(3) Fig. 1A-D: Was these images obtained by whole mount labeling of anti-E-cadherin antibody? Please cite references which provide the information on this antibody. Furthermore, the authors did not mention the orientation of lenses in figure legends. Since the retina is located in the left side, so anterior direction is right side. Please indicate the orientation of the lens in figure legends.

Thanks and we have added the orientation information in Fig. 1A for Confocal images, and the information in Fig. 1G for TEM images. Now all confocal images including Fig. 2A and 2B are in the orientation same with Fig. 1A except Fig. S4 (the live imaging). All TEM images of lens epithelia are in the orientation same with Fig. 1G except Fig. S1 (matching to the figure configuration). All Confocal images were stained with antibody after cryosection except Fig. S4 (live imaging). The detail information of the anti-E-cadherin can be found on the provider's website (Catalogue number: BD transduction 610182). We now provided a reference for its application in zebrafish in Methods.

(4) There is no description of EM data shown in Figure 1E-G in the results section. Every figure panels should be cited in the text.

Thanks and we have accordingly re-edited Fig. 1G and revised the text. Please see comments 7 for

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the revision of Fig. 1G and 1H.

(5) Page 4, line 4: Abbreviation of "WT" should be shown as "wild type (WT)" at the first place.
Thanks and we have accordingly revised the text.

(6) Fig. 1K, 1M was not cited in the main text of results section.
Thanks and we have accordingly revised the text.

(7) Contrast of Fig. 1E and 1H is not good. Please improve it.
Fig1E and Fig1H in the previous version are the lower resolution TEM images (1200X) to show the position of Fig1F-K. A 1200X resolution of TEM images are always fuzzy and uneven brightness. Thus we replaced these two images with schematic drawings and moved them to Fig. S1.

(8) Orientation of Fig. 1G should be consistently assigned with box 2 of Fig. 1E.
Thanks and we have accordingly re-edit Figure 1 combining 3 reviewers' comments.

(9) Legend of Fig. 1. (E-G): The sentence "The black boxed region of I shows the apical zone between two epithelia" may be mistaken to be inserted into legend of (E-G), but should be moved into the right place of legend (H-J).
Thanks and we have accordingly revised the text.

(10) Page 4, line 17: The term "dissolved" may be changed into "disassembled".
Thanks and we have accordingly revised the text.

(11) Fig. 1I: It is difficult to see the outline of lens epithelial cells and lens fiber cells. Please provide a schematic drawing. Fig. 1J orientation should be consistent with box 2 of Fig. 1H.
Thanks and we have accordingly re-edit Figure 1 combining 3 reviewers' comments, added the orientation information, and colored all lens epithelia in Fig. 1-6 to show the outline of the cells.

(12) Figure panel configuration is not good in Fig. 1. EM image panels at the same stage ((E, F, G) at 24 hpf and (H, I, J, K) at 36 hpf) should be assembled together, for example, aligned in the same horizontal row. Furthermore, panel size and outline also should be consistently organized.
Thanks and we have accordingly re-edit Figure 1 combining 3 reviewers' comments.

(13) Scale bars should be shown in Fig. 1E and 1H.
Thanks and we have accordingly re-edit Figure 1 combining 3 reviewers' comments.

(14) Orientation of lens is obscure in Fig. 2A. Fig. 2A seems to be obtained from the anterior view (lateral view of the eye), although Fig. 2B seems to be obtained from the ventral view of embryo. Please make sure the orientation of lens consistent. Furthermore, there is Nok signal in central part of the lens at 28 hpf (Fig. 2B). Is this a real signal or noise? Please carefully set the signal threshold.
Thanks for the reviewer's comments and suggestions. Fig. 2A and Fig. 2B are in the same orientation. Now we replaced the Fig. 2A. The Nok signal in central part is background signal. This signal was shown because the real Nok signal at the apical regions is also weak at this point (28 hpf, at the beginning of epidermal-to-epithelial transition).

(15) Fig. 2F: The increase in number of apical AJs between epithelia-fiber cells in nok mutants may be due to invasion of epithelia into inner lens. This should be mentioned in the text. How about the AJ formation between ingressed (Zl1-netative) lens epithelial cells and Zl1-positive lens fiber in nok mutants?
Thanks and we have accordingly revised the text. When examining the AJs by TEM (cannot stain with Zl1), we cannot distinguish the Zl1-netative ingressed cells from Zl1-positive fiber cells. In another side, confocal imaging (with Zl1 staining) does not have the proper resolution to examine AJs organization.

(16) Please mention results on the length of AJs in nok mutants in the main text by citing Fig.

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2G. It may be better to merge Fig. 1L and 2G, because only length of AJ_EE (Fig. 2G) is shorter in nok mutants than in wild type (Fig. 1L).
Thanks and we have accordingly re-edited the figure and revised the text.

(17) There is no citation on Fig. S3 panels (A, B, C, G, H) in the main text.
Thanks and we have accordingly revised the text.

(18) Fig. S3 (FGH): Please explain why lens fiber cell number is higher in nok mutants than in wild type at 36 hpf in the main text. If the authors count nuclear number of fiber cells, denuleation (degradation of lens fiber cells) may be compromised and slower in nok mutants.
Thanks for the reviewer's comments and suggestions. The ingressed epithelia eventually differentiated into lens fiber cells, resulting in an increase of the number of lens fiber cells (Figure S4F-S4H). We have now re-edited Fig. S4 and added the explanation. Denucleation in zebrafish lens fiber cells began after 48 hpf (Greiling et al., 2009), and we can see nuclei staining in each fiber cell at this stage (combining actin staining and Dapi staining), thus denucleation should not be involved in this stage (36 hpf).

(19) Fig. 3A-F: Please cite literatures that used the rab11 antibody in zebrafish. There are Rab11 antibody signals in lens of rab11a and rab11ba mutants, and also double mutants, so I wonder which rab11 subtypes are recognized by this antibody or whether the epitope site of this antibody is located in the N-terminal region of premature stop of the rab11a and rab11ba mutants, which the authors generated by Crispr/CAS9. Please provide the more detailed information on this antibody.
Thanks for the reviewer's comments and suggestions. The polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg184 of human Rab11a protein. The truncated rab11a protein in rab11a^{ZJUKO233} contains amino acid 1-59, and the truncated rab11ba protein in rab11ba^{ZJUKO234} contains amino acid 1-92. Given the high identity among Rab11 subtypes, the antibody may recognize all Rab11 proteins (such as Rab11a, Rab11ba, Rab11bb). We have accordingly added the information for the truncated proteins in figure legends (Fig. S5) and for the antibody. We did not find the immunohistochemistry application of this antibody in zebrafish. However, our results in rab11 knockout animals indicated that the antibody worked well in zebrafish.

(20) Fig. 3G-K: In accordance with materials and methods, this series of experiments seemed to be done using transient expression of GFP-tagged rab11 by injection of DNA construct. Please mention it in the main text. In addition, there is no description about how Rab11a Q70L-eGFP expression was introduced in materials and methods. Please revise it.
Thanks and we have accordingly added the information in Methods/ In vitro transcription of RNA and micro-injection section and in Figure legends.

(21) Fig. 3K: The authors mentioned that Rab11a S25N severely impeded the apical localization of Nok; however, actual phenotypes were weak. Please conduct statistical analysis and show its histogram.
Thanks for the reviewer's comments and suggestions. The negatively regulation of Rab11a S25N on Nok localization is dose-dependent. We now provided new data for the higher level over-expression of Rab11a S25N (Fig. 3O, by plasmid injection), provided new data by Rab11a S25N-2A-eGFP mRNA injection (Fig. 3K and 3L), and re-edited the control images (Fig. 3M, over-expression of WT Rab11a). Comparing to controls (WT zebrafish in Fig.2C, and over-expression of WT Rab11a in Fig. 3K and M), we can clearly see the significant differences: sporadic expression of Rab11a S25N at lower level induced the lateral and basal enrichment of Nok in lens epithelia (Fig. 3N). More importantly, with higher level of Rab11a S25N, the apical signal of Nok was almost completely lost in lens epithelia (Fig. 3L and 3O). Together with the results from loss-of-function shown in Fig. 3G-3J), we can make a conclusion that Rab11 regulates the apical localization of Nok in lens epithelia. Because the dose-dependent effects, we did not do the statistical analysis for figure 3N. Please also see in reviewer 1's comments.

(22) Fig. 3: Please show labeling of Nok antibody in rab11a/rab11ba mutants, whose signal is expected to be absent.
Thanks and we have accordingly performed the experiment (Fig. 3J). Nok signal is absent in rab11a/rab11ba mutants.

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(23) Fig. 3 panel configuration: vertical outlines of panels (A, A', D, D') and panels (G, G', H, H') should be aligned.
Thanks and we have accordingly revised the text.

(24) Fig. 4K and 4L: Overlapping of rab11, Nok, and GM130, a Golgi marker or PDIA3, an ER marker was not clear. Please show higher magnification images. Statistical analysis is also required to show the percentage of triple positive foci in rab11a-positive foci, Nok-positive foci or GM130-positive foci. It is ideal and practically possible to conduct the same experiments using zebrafish lens epithelium, rather than human in vitro culture.
Thanks for the reviewer's comments. We have shown the higher magnification images (Fig. 4M and 4P) and performed the Statistical analysis (Fig. 4N). Unfortunately, we failed to isolate and generate a zebrafish lens or retinal epithelium line. Furthermore, the endogenous Rab11 and Nok proteins could not be detected in cytoplasm, probably due to the quick exocytosis of nascent proteins after translation in living zebrafish. Similar with the endogenous proteins, the over-expression of Nok proteins could not be detected in cytoplasm. Thus, we performed the experiments in MDCK cells, a classic epithelial cell line widely used to mimic mammalian epithelia tissue in the cellular polarity and adhesion field (Simmons, 1982; Chavrier et al., 1990; Hurd et al., 2003; Li et al., 2014; Mrozowska and Fukuda, 2016). Please also see reviewer 1's comments.

(25) Fig. 4K: In contrast to nok mutants, number of plaques of AJ_EF is not different between wild-type and rab11 single mutants. This may be due to normal monolayer of lens epithelium in rab11 double mutants. However, number of plaques of AJ_EF is reduced in rab11 double mutants. Please explain why.
Thanks for the reviewer's comments. Compared with WT, in single rab11a or rab11ba mutants, the apical localization of Crb complex was partially affected (Fig. 3G and 3H), and the number and average length of AJs_EE were partially affected (Fig. 5K and 5L), suggesting the functional redundancy of Rab11a and Rab11ba in lens epithelia. Supporting this, in rab11a/rab11ba double mutants, both the number and average length of AJs_EE were significantly decreased.
Double knock out of rab11a and rab11ba induced a significant decrease of AJs_EE, suggesting a critical role of Rab11 in AJs_EF formation. Different from AJs_EE, single rab11 knockout did not induce a significant change of AJs_EF. We think it likely caused by the redundant function of Rab11a and Rab11ba in zebrafish lens epithelia. However, due to lack of proper model, we cannot identify the precise role of endocytosis and exocytosis in the formation of AJs_EE and AJs_EF. Given that Rab5 S36N did not affect the localization of Nok, we cannot exclude the possibility that AJs_EE is mainly regulated by Rab11-medated exocytosis, and AJs_EF is mainly regulated by Rab11-medated recycling.
We have accordingly revised the results and discussion.

(26) Fig. 6: The authors conclude that Par complex does not recruit rab11. Please indicate rab11a expression in aPKC mutants. It is interesting to investigate whether apical localization of rab11a is independent of aPKC.
Thanks for the reviewer's comments. We have added the new data and accordingly revised the text. In aPKCλ^{m567} mutants, the immunostaining signal of aPKC is lost, while both Rab11 and Crb2a signals are co-localized at the apical regions in some epithelia (Fig. 6H and 6I). Given that most of Crb2a proteins lost their apical localization in aPKCλ mutants, it is expectable that most of Rab11 lost their apical localization.

(27) Fig. 6G and 6H: It is better to show the data of lens epithelium rather than surface epidermis. Is a similar tendency of cytoskeleton organization observed in Fig. 6F?
Thanks for the reviewer's comments. The filaments is only visible by TEM in surface epidermal cells (Fig. 6F and 6G), but not in lens epidermal or epithelial cells (Fig. 1E and 1G), probably adapting to the dynamic cell state transition during lens development.

(28) Fig. 6 panel configuration should be improved. Location of panels (B-B") is not good. Panels A-C should be vertically aligned together. Interval space between panels E/F, G, H can be smaller, then panel J will be moved into the right side.
Thanks and we have added new data in this figure and accordingly re-edited the figure configuration.

(29) Legend of Fig. S1: Scale bar 20 um -> 20 micro-m (font change)

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Thanks and we have accordingly revised the text.

(30) Fig. S1C: Authors mentioned that arrowheads indicate non-specific signals. What tissues does the antibody non-specifically bind in the basal region of wild-type retina (blood vessels?)? Furthermore, are signals in the outer neural retina of the nok mutants non-specific?
Thanks. We think the reviewer mentioned Fig. S2C. The non-specific signals are occasional and we are not sure what it is. We have accordingly replaced the images.

(31) Fig. S1F: Focus is not good and blur in these images. I suggest the authors to take image using a compound microscope, but do not enlarge images obtained by a dissecting microscope.
Thanks. We think the reviewer mentioned Fig. S2F. We have accordingly replaced the images.

Reviewer 3
Monday 23rd of September 2019

Development
Manuscript number: DEVELOP/2019/184457
Title: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens
Authors: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian A. Link, Ke Yao, Jian Zou

In this paper, Hao and colleagues analysed the interaction between the Crumbs-MPP5a-PATJcomplex (Crb complex) and the small GTPaseRab11 during the establishment of apical cell polarity in the developing lens and retina of the zebra fish. Through this interaction, the vesicle exocytosis is biased towards the apical cell domain. Interfering with either with the function of the Crb complex or the function of Rab11 results in ectopic localisation of Crb and adherens junction (AJ) components.
The authors nicely showed in vivo that the AJs are progressively established during lens development. This gradual formation of proper AJ depends on the gradual apical localization of the Crb complex.
Hao et al. produced a great amount of work in generating nok and rab11mutant lines using the CRISPR/Cas9 technique. They also did some live imaging of lens development in these mutant contexts.
In addition, they performed a detail analysis of AJ formation in various genetic contexts by transmission electron microscopy. However, they do not take the best advantage of this analysis, as the panels are far too small and not well organised within a figure. For example in Figure I, it is very hard to spot the two squares 1 and 2 in panel E. These two areas are enlarged in panels F and G respectively. It would be of great help to have the three panels on the same line rather than to browse throughout the figure. The same stands true for panels H-K. Panel layout is also very confusing in Figure 4.
In spite of this, it is already known in many other systems that Rab11ditribution is apically bias to fully support secretion of AJ components. In addition, I don't think that authors reach their conclusions with the data they provided.
Thanks for the reviewer's comments. We have added new data and re-edited Figures accordingly (combining with reviewer 1's and 2's comments).

Previous studies have reported that Rab11 enriches at the apical domains in epithelia and play an important role in secretion of AJ components (Stenmark, 2009; Apodaca et al., 2012; Woichansky et al., 2016). Also, it has been well known that zonula adherens lost in the mutant with apical polarity defects (Bulgakova and Knust, 2009; Tepass, 2012; Chen and Zhang, 2013). However, it is largely unknown yet how to drive the Rab11-mediated vesicle exocytosis to the apical domains in epithelia, and the molecular mechanism through which the apical polarity proteins regulate zonula adherens formation. In this study, our major conclusion is that we identified an unrecognized MPP5a/Rab11 complex and described its essential role in guiding apical polarization and zonula adherens formation in epithelia. Furthermore, we observed that Par complex recruited by MPP5a is incapable to interact with Rab11 and described a phenotype that aPKC may assemble cytoskeleton to facilitate the cadherin exocytosis. We provided genetics (both knock-out and dominant negative over-expression animal models, and proper controls) and cellular/biochemical evidences to support that Rab11/Nok interplay synergistically builds the apical polarity and zonula adherens in epithelia. We believe this conclusion is an interesting and substantial advancement for an important issue in

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the field. Now we added more evidences to strengthen our conclusion. We agree that many questions, such as how endocytosis pathway regulates the apical AJs (including apical zonula adherens between lens epithelia-epithelia and punctum adherens between epithelia-fiber cells) in vertebrate, and how aPKC regulate the orientation of cytoskeleton, are very interesting for future studies. Please see the revised manuscript for the details and see below for our point-to-point responses.

1- From Figure 3, they concluded for a reciprocal dependency of Rab11 and the Crb complex. They used a Rab11a-eGFP reporter line to follow Rab11 distribution in a nok mutant context. But this reporter line expression profile (panel G') does not resemble the endogenous Rab11 distribution.
Thanks for the reviewer's comments. The data to support our conclusion include both the Rab11 reporter line expression (Fig. 3D-3F, 3K-3P) and the endogenous Rab11/Crb2a and Nok expression (Fig. 3A-3C, 3G-3J and 6J). Both of these models support a reciprocal dependency of Rab11 and the Crb complex. For the localization of endogenous proteins, we observed, 1), in WT, Rab11 and Nok were simultaneously and exclusively enriched at the apical domains in epithelia during epidermal-to-epithelia transition, but not in the cells at other stages such as lens epidermal and mesenchymal cells (Fig3A-3C). 2), In nok mutants, Rab11 lost its apical enrichment in lens epithelia. Reversely, in rab11a/11ba double knockout mutants, Crb2a and Nok lost their apical enrichment in lens epithelia (Fig. 3G-3J and 6J).

2- The authors used HEK293T cells to perform co-immunoprecipitation and co-localisation assays. These cells are human cells derived from human kidney, transfected by a variety of proteins of zebra fish or human origin. I find it very confusing to ascertain with protein interacts with another one independently of the cell context. In addition, Hao et al concluded that the Crb complex interacts with Rab11in the Golgi apparatus. However, they drew their conclusion from cells that do not seem To have form AJs.
Thanks for the reviewer's comments. We are sorry for the misleading writing. We performed co-IP experiments (Fig. 4A-4C) and the membrane associated β-catenin intensity experiments (Fig. 4F-4J) in HEK293T cells transfected by a variety of zebrafish proteins, and performed endogenous co-IP and the co-localization of Rab11/Nok/GM130 (Golgi marker) in MDCK cells. HEK293T cells do not have typical apical polarity and zonula adherens like that in epithelia, but do express N-cadherin and β-catenin (very likely, organizing into punctum adherens) (Inada et al., 2016; Zou et al., 2012). Thus, we examined whether the introduction of exogenous Crb2a/Nok proteins could remodel the AJs distribution in HEK293T cells or not. MDCK cells have zonula adherens and Crb/Pals1 expression, and are widely used to mimic mammalian epithelia tissue in the cellular polarity and adhesion field (Simmons, 1982; Chavrier et al., 1990; Hurd et al., 2003; Li et al., 2014; Mrozowska and Fukuda, 2016). Thus, we examined the co-localization of Rab11/Nok/Golgi in MDCK cells. Now we performed new experiments to prove the interaction between Rab11 and Nok by endogenous co-IPs using zebrafish eye extract and MDCK cells extract (Fig. 4D and 4E). Surely, it would be the best if we can observe the co-localization in living animal. Unfortunately, the endogenous Rab11 and Nok proteins could not be detected in cytoplasm, probably due to the quick exocytosis of nascent proteins after translation in living zebrafish. Similar with the endogenous proteins, the over-expression of Nok proteins could not be detected in cytoplasm. Please also see reviewer 1's comments. We have revised figure legends to clearly show the cells we used in the experiments and accordingly revised the text.

3- Finally, authors jump on the relationship between the Par complex and the actin cytoskeleton. They showed that the Par complex enrichment is independent of the Crb/Rab11complex localisation. They controlled that the Par complex may control the orientation of the actin cables, as actin fibres change orientation in a PKC mutant background.
At the resolution displayed on the figure, this is quite impossible to conclude anything about actin fibres orientation and the transmission electron microscopy is not helpful.
In addition, I don't think the authors can reach such strong conclusion, as it can be an indirect consequence of affecting another cytoskeleton. It would be very helpful is Hao et al. could directly disturbed the actin cytoskeleton and assed AJs remodelling.

Thanks for the reviewer's comments. The TEM resolution could be more than 0.2nm. Among three major types of cytoskeleton, the diameter of microtubules is about 25nm, the diameter of intermediate filaments is usually about 10nm, and the diameter of microfilaments (actin filaments) is usually several to 10nm. Thus, the actin filaments should be theoretically visible by TEM, and

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electron microscopy (EM) is a key tool to determine the structure of the cytoskeleton (Svitkina et al., 1995 and 2009). Indeed, one important marker to distinguish zonula adherens from punctum adherens is the orientation of actin filaments visualized by TEM (Yonemura 2011). In this study, we indeed observed by TEM the filaments with a several nm diameter which is similar within the reference in surface epidermal cells (Fig. 6F and 6G), but not in lens epidermal or epithelial cells (Fig. 1E and 1G), probably due to the dynamic cell state transition of lens cells during lens development.

We have removed unpublished data provided for the referees in confidence.

Previous studies have reported that Cytoskeletal modulation is also involved in the vesicle trafficking (Lanzetti, 2007; Horgan and McCaffrey 2011), and aPKC regulate the cytoskeletal proteins including both actin and microtubule (Uberall et al., 1999; Betschinger et al., 2003; Harris and Peifer, 2007; Hosono et al., 2015). Consistent with previous reports, we observed that actin enrichment is associated with aPKCλ by immunohistochemistry and confocal imaging (Fig 6H-6L). In this study, the new phenotype that we described is that the orientation of cytoskeleton was changed in aPKCλ^{m567} mutants (revealed by TEM). However, we did not make a conclusion which types of cytoskeleton was affected in this study, given the focus of this study is on Rab11/Nok complex. We agree that it would be interested to further analyze how aPKC regulates cytoskeleton orientation in future studies.

4- Final minor point, the figures are too small, close up will be very appreciated. In addition the schematic representation of AJ distribution is far too small. It is nearly impossible to spot the legend on the illustration. In addition, the confocal images display the developing lens on the right hand-side while the drawing shows it on the top. Why not adopting the same position?
Thanks for the reviewer's comments. We have accordingly re-edited the figures (combining the reviewer 1 and 2's comments).

In conclusion, I don't think this work raises the novelty high enough to fully support publication in Development and I recommend publication in a more specialised journal.

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Second decision letter

MS ID#: DEVELOP/2019/184218

MS TITLE: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens
AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, Jiancheng Liang, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees still have some criticisms and concerns that must be addressed before we can consider publication. Reviewer 3 suggests that some data is weak and should be removed but it would be better if you could address the concern by strengthening the data rather than removing it.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

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Reviewer 1

Advance summary and potential significance to field

In this revised version, Yumei Hao and colleagues have improved their manuscript 'Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens'. The authors provide new data sets and have discussed comments and questions submitted by the reviewers. Nevertheless, there are still important points that I believe need to be clarified before publication.

Comments for the author

Main points

1: The apical localization of Nok and Rab11 is reciprocally dependent

The effect of nok mutant upon Rab11 reavealed by antibody labelling is clear (Fig 3 B-B' and Fig 3 C-C'). However, the results obtained with Rab11-eGFP are difficult to interpret. The apical enrichment of Rab11 a-e-GFP is difficult to follow in wild-type (Fig 3 M), hence it is difficult to assess the absence of Rab11 a-e-GFP apical distribution in nok mutant. I have the same comment for Rab11Q70L-GFP (Fig 3 E and Fig 3F). The authors should clarify these points and modify the text accordingly.

2: Par complex may organize cytoskeleton to facilitate the vesicle exocytosis, but not via direct recruitment of Rab11

I have problems with the interpretation of the results for Figure 6 and more specifically from line 296 with the part "We also revealed that the localization of Rab11 was associated with Crb complex but not aPKC (Fig. 3C, 6J, and 6L). Intriguingly, different from the Crb complex, the apical enrichment of aPKC does not rely on Rab11 (Fig. 6J and 6L)."

First: In figure 6 the distribution of Rab but also that of Crb in a mutant context for PKc is very strongly different from that observed in a wildtype context with figure 3B; Also for me, the distribution of Rab11 and Crb is affected in a Pkc mutant context.

Second: The way the quantification was done for Figure 6L is difficult to understand. The quantification should compare the apical and basal distribution in nok mutants versus wild-type. Moreover, the results of the fig6K pointing to an heterogeneity in the apical localization defect for apkc should be more discussed in the result and not only shortly mentioned in the legend of the figure.
This heterogeneity for Pkc apical localization of between anterior and lateral epithelial cells is also an issue for the quantification figure L made from the data of the figure 6K. How is this heterogeneity taken into account for quantification?

Third : Relationships between the actin cytoskeleton Crumbs and PKc

On the basis of the results presented, it is very difficult if not impossible to draw conclusions concerning the actin network, Crb and PKC. Moreover the comparison between what can be an actin network between figures 6 F' and 6G' is very difficult because the scale of the figures is not the same and if one enlarges figure F' it seems that one also finds fibers with the orientation of the fiber in G'.
Thus the conclusions concerning the links between the active network, PKC and Crb should be on my opinion removed from the results. Furthermore, the title of this part of the results needs to be changed as it is not supported by the results.

Minor points
In the Figure S1 panel E, the scale bar is in mirror image

In the result section line 191"In detail, either sporadic over-expression generated by plasmid injection (Fig. 3M) or overall over-expression generated by mRNA injection (Fig. 3O) of Rab11a did not significantly affect the apical enrichment of Nok in WT lens epithelia.

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I think, the reference to Fig. 3O should be replaced by the reference to figure 3K.

Reviewer 2

Advance summary and potential significance to field

In this manuscript, the authors reported that zebrafish Stardust homologue Nok interacts with rab11 to promote exocytosis of AJ components, casdherin and Crumbs, leading to the establishment of the apical domain polarity and adherens junction in zebrafish lens epithelium. The authors also found that Nok recruits Par complex, which does not interact with rab11 but assembles cytoskeleton to support the exocytosis of adherens junction components. These data suggest a novel role of rab11 and Nok in the formation of adherens junction of zebrafish lens epithelium.

Comments for the author

I have reviewed the revised manuscript. The authors have responded to my suggestion. In accordance with my suggestion (20), the authors have added the description on how EGFP-tagged rab11 expression is introduced. However, the authors did not mention how to prepare DNA constructs expressing EGFP-tagged rab11a and EGFP-2A-rab11a in Materials and Methods; rather they simply stated that the DNA constructs:
Tol2[EF1a:rab11a-EGFP], Tol2[EF1a:rab11a(S25N)-EGFP], Tol2[EF1a:rab11a(Q70L)-EGFP], Tol2[EF1a:rab5(S36N)-EGFP] as well as pCS2[CMV promoter(?): EGFP-2A-rab11a] and pCS2[CMV promoter(?): EGFP-2A-rab11a(S25N)] were used for experiments shown in Figure 3D-F; 3K-P, Fig. S6 and Fig. 4IJ.

My concern is whether Tol2[EF1a:rab11a-EGFP] expresses C-terminal EGFP-tagged rab11a or N-terminal EGFP-tagged rab11a. My understanding is that the C-terminal cysteine containing domain of small G-proteins including rab5 and rab11 is important for membrane anchoring, and that localization of rab5 and rab11 to early and recycling endosome (or TGN) is critical for their functions. Thus, N-terminal EGFP tagged rab proteins are generally used for such an overexpression experiment. Although one reference the authors cited (Chen et al., 1998) used N-terminal EGFP-tagged rab11, it is obscure whether the authors used N-terminal EGFP-tagged rab11/rab5 in their expression constructs. If the authors used C-terminal EGFP tagged rab11/rab5, interpretation of data on Figure 33D-F; 3K-P, Fig. S6 and Fig. 4IJ is difficult. In general, N-terminal GFP-tagged rab11 and rab5 signals are usually observed as dotted signals corresponding to recycling endosomes and early endosome respectively, both overexpressed rab11-EGFP and rab5-EGFP signals were likely to be more broadly observed in cytoplasmic region of lens cells in Fig. 3D-F; 3M-P. Thus, I wonder if the authors used C-terminal EGFP tagged rab11 and rab5 for these overexpression experiments. Please confirm that N-terminal EGFP-tagged rab11 and rab5 were used, and state DNA construction procedures in detail in Materials and Methods. If the authors used N-terminal EGFP-tagged rab11, the authors must state “Tol2[EF1a: EGFP-rab11a]”.

On the other hand, overexpression data using pCS2[EGFP-2A-rab11a] and pCS2[EGFP-2A-rab11a(S25N)] (Figure 3KL) are acceptable from experimental design point of views. However, Fig. 3KL showed the indication of “rab11a-2A-EGFP” and “rab11a(S25N)-2A-EGFP”, which also causes confusion: I wonder which “EGFP-2A-rab11a” or “rab11a-2A-EGFP” the authors introduced into lens. In addition, Fig. 3 legend (see the line 733) stated that rab11a(S25N)-2A-EGFP was overexpressed in lens (N and O), which is inconsistent with figure panel indication in using rab11a-EGFP and rab11a(S25N)-EGFP. Please check your experiment designs and data of Figure 3 as well as text description in your manuscript.

If the authors unfortunately used C-terminal EGFP/mCherry-tagged rab11 or rab5, my suggestion is that the authors should delete all the data on Figure 3DEF, 3MNOP; rather the authors should reexamine using N-terminal EGFP-tagged rab11/5 or conduct experiments to show Crb2 protein expression of wild-type lenses injected with mRNA of EGFP-2A-rab11a and EGFP-2A-rab11a(S25N), as well as experiment using EGFP-2A-rab5 (S36N) mRNA. The authors also need to reevaluate reciprocal dependency between Rab11 and Crb complex in retinal neuroepithelia (Fig. S6), and in vitro expression data (Fig. 4IJ, and 4LMOP), using N-terminal EGFP/mCherry tagged rab11 constructs.

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Another point that I would like to request is that figure panels should appear in the alphabet order in the main text of the results. For example, as Fig. 1 panels apprear in the main text as 1AB->1EF->1CD; Fig.3 as 3DEF->3M-> 3GH; Fig. 6 as 6EFG->6JL-> 6HI-> 6K; Fig. S3 as S3A-D->S3G->S3EF). In addition, three supplementary figures S2, S3, S4 hang on Figure 2, and citation order of these supplementary figures is also intermingled across S2-S4 in the text, which makes us difficult to follow the logics of results. Please revise in the text or change panel configuration of Fig. S2-4 as well.

The third, I would like to request the authors to revise the discussion. There are several mistakes. For example, line 337: Fig. S5A-> Fig. S6AB; line 379: out->our. Some sentence such as line 336-338 need to be revised grammatically.

Specific comments
(1) Line 110: Does "the intersection foci of cells" mean the vertex point where three neighbor cells meet? Fig. 1A does not show the apical plane of lens epithelium because epidermal to epithelial transition does not occurs at 24 hpf. So, the authors may mention simply that E-cadherin is observed as foci, which are generally associated with plasma membrane, especially enriched at the vertex where three neighbor cells meet.
(2) Line 721-722: Do not insert the explanation sentences on other figure panels in Figure 1 legend.
Orientation of lens should be described in each figure legends.
(3) Line 147: the lateral interfaces between -> the basal and lateral interfaces between
(4) Line 175-179: Crb2a signals are not observed in Fig. 3E; it is also difficult to tell that the constitutive active form of rab11-EGFP signals is localized in apical domain of WT lens epithelium from the images of Fig. 3E; it is also difficult to tell that rab11-EGFP signals are localized in the apical domain of WT lens epithelium from the image of Fig. 3M. Thus, please replace these images with more convinced ones. I suggest the authors to show more higher magnification images.
(5) Line 183: Fig. S4-> Fig. S5
(6) Line 193: Fig. 3O -> Fig. 3K
(7) Line 296: absence -> absent

Reviewer 3

Advance summary and potential significance to field

Hao and colleagues have now added new data and clarifications to the current version of this manuscript. I greatly appreciate the efforts the authors took to discriminate the functions of Rab5 and Rab11 during exocytosis and endocytosis. I also appreciate their effort to perform co-IP using not only mammalian cell extracts and also fish eye extracts.

Comments for the author

However I have two major problems that remain.

1. They authors either in their response letter and in the revised manuscript reach " the conclusion that the localization of Rab11 is associated Crb2a with but not actin; and the localization of actin is associated with aPKC, but not Crb2a ».
This conclusion is based on the observations: "In aPKCλm567 mutants, the immunostaining signal of aPKC was lost, actin also lost its apical localization; however, both Rab11 and Crb2a signals were co-localized at the apical regions in some epithelia (Fig. 6H and 6I). In nok mutants, the immunostaining signals of both Rab11 and Crb2a were lost in all lens epithelia, while both aPKC and actin were enriched at lens lateral epithelia (Fig. 3C, 6K, and 6L)"
This conclusion bothers me because I don't think the data support it. I am afraid that the authors force too much on this conclusion because the co-IP experiments did not show an interaction between Rab11 and aPKC.
Hao et al. wrote "Rab11 and Crb2a signals were co-localized at the apical regions in some epithelia". However, in Figure 6I, the distribution of Rab11 seems to be strongly affected and that of Crb2a very disturbed.

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Similarly, in Figure 6K, the aPKC and actin stainings are affected in a nok mutant background.

2. Hao and colleagues wrote in the manuscript: “We observed that the apical enrichment of F-actin is closely associated with aPKC localization in lens epithelia, rather than localization of Crb complex (Fig. 6D, 6I, and 6J). Cytoskeletal components were organized perpendicularly to the cell-cell contact surface in WT surface epidermis cells, but were oriented in parallel in aPKCλ mutants (Fig. 6G and 6H).”
I don't think the authors have sufficient resolution using confocal microscopy to reach this conclusion. I have even more problems with TEM.
In WT context, Hao and colleagues show perpendicular actin fibres relative to the cell-cell contact surface. These fibres are at a certain distance from the cell membrane and the adhesion plaque. In Figure 6F', the size of an adhesion plaques is 3mm.
In a mutant context for aPKC, the authors detect actin fibres immediately parallel to the adhesion plaques and do not show anything at the same distance as in the WT situation. However, at the right end of the figure, do we not see parallel actin fibres?
Moreover, on Figure 6G', the size of an adhesion plate is 4 mm. Clearly Figures 6F' and 6G’ are not at the same resolution even if the scale bar would tend to make us believe it.

In conclusion, Hao and colleagues produced a great amount of work in demonstrating the novel role of Nok/Rab11 complex in the apical polarity establishment.
I would not recommend adding the data relative to aPKC/actin as they don’t appear robust enough. I would then recommend rewriting the summary accordingly. Under these conditions, it remains to be decided whether this dataset is sufficiently new to justify publication in Development.

Second revision

Author response to reviewers' comments

Dear Editors and reviewers,
We thank the editors and reviewers for their efforts in evaluating our manuscript again and their constructive comments. In this version, we have replaced the Fig. 3D and Fig. 3E, reedited the order of panels, and revised the text (marked with blue color in the manuscript) according to the reviewers’ comments and suggestions. We believe that all concerns/questions raised by the reviewers have been addressed (except no new data for the TEM observation of cytoskeleton, shown in Fig. 6F and 6G in old version), and the revised manuscript is significantly improved.
In this study, we elucidated that Nok and Rab11 form a novel complex in epithelia. The interplay between Nok and Rab11 guides the apical exocytosis to synergistically establish the apical polarity as well as the formation of zonula adherens during the maturation of epithelia. In the study, we also observed some interesting phenotypes, such as the heterogeneity for the association between Crb-MPP5 complex and Par6-aPKC complex in different lens epithelia, and the cytoskeleton orientation modulated by aPKC (please see detail in our responses to reviewer 3’ comments). Due to the ongoing COVID-19 epidemic disease, all of labs at Zhejiang University have been temporarily closed by Chinese government since early February 2020. Be subject to this law, during the time (early January to now), we could not do any new experiments to provide more data to elucidate the detail mechanisms on the heterogeneity and cytoskeleton modulation.
Please check below (marked with blue color) for our point-by-point responses to the reviewers' comments:

Reviewer 1 Advance Summary and Potential Significance to Field:
In this revised version, Yumei Hao and colleagues have improved their manuscript “Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens”. The authors provide new data sets and have discussed comments and questions submitted by the reviewers. Nevertheless, there are still important points that I believe need to be clarified before publication.
Thanks for the reviewer's comments and suggestions. Please see below for our point-to-point responses.
Reviewer 1 Comments for the Author:

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Main points

1: The apical localization of Nok and Rab11 is reciprocally dependent

The effect of nok mutant upon Rab11 revealed by antibody labelling is clear (Fig 3 B-B' and Fig 3 C-C'). However, the results obtained with Rab11-eGFP are difficult to interpret. The apical enrichment of Rab11a-e-GFP is difficult to follow in wild-type (Fig 3 M), hence it is difficult to assess the absence of Rab11a-e-GFP apical distribution in nok mutant. I have the same comment for Rab11Q70L-GFP (Fig 3 E and Fig 3F). The authors should clarify these points and modify the text accordingly.

Thanks for the reviewer's comments. The immunohistochemistry data to prove the reciprocally dependent localization between Nok and Rab11 include two sets. The most important set is the reciprocally dependent localization of endogenous Nok and Rab11 proteins in wild-type and mutants, which is acceptable for the reviewer.

Another set is the overexpression of Rab11a and its mutants. When eGFP-Rab11 is expressed too much in lens epithelia, the localization of eGFP-Rab11a could be spread to other regions except apical domain such as the lateral regions, however, it is clear that the eGFP intensity at the apical regions is much higher than the other regions in wild-type embryos (Fig 3M in the old version). At the proper level of overexpression, we observed that both eGFP-Rab11a and eGFP-Rab11a Q70L (the constitutive active mutation) was localized at the apical domain (Fig 3D and 3E in this version). Reversely, in nok mutants, we observed that eGFP-Rab11 and eGFP-Rab11 Q70L are spread to the apical, lateral and basal at comparable eGFP intensity (Fig 3F and 3G). Furthermore, eGFP-Rab11 S25N (the dominant negative mutation) are spread to the apical, lateral and basal at comparable eGFP intensity in wild-type embryos (Fig 3N and 3O). Thus, we believe we can make the conclusion that the apical localization of Nok and Rab11 is reciprocally dependent.

We have replaced the Fig3E and 3M (old version number) and accordingly revised the text to provide more detail description (line 179-181).

2: Par complex may organize cytoskeleton to facilitate the vesicle exocytosis, but not via direct recruitment of Rab11

I have problems with the interpretation of the results for Figure 6 and more specifically from line 296 with the part "We also revealed that the localization of Rab11 was associated with Crb complex but not aPKC (Fig. 3C, 6J, and 6L). Intriguingly, different from the Crb complex, the apical enrichment of aPKC does not rely on Rab11 (Fig. 6J and 6L)."

First: In figure 6 the distribution of Rab but also that of Crb in a mutant context for aPKC is very strongly different from that observed in a wildtype context with figure 3B; Also for me, the distribution of Rab11 and Crb is affected in aPKC mutant context.

Thanks for the reviewer's comments. Both Crb-MPP5 and Par6-aPKC-Cdc42 are known as the key apical polarity complexes. Dysfunction of either complex causes the mislocalization of another complex and the loss of cellular polarity in epithelia (Bulgakova and Knust, 2009; Tepass, 2012; Chen and Zhang, 2013). In this study, we aimed to clarify the distinct function between these two complexes. We concluded that Nok (MPP5a in zebrafish) formed a complex with Rab11 to guide the apical exocytosis of cadherin molecules during adherens junction remodeling. Given dysfunction of aPKC could affect the apical localization of Crb complex, it is reasonable that aPKC indirectly, but not direct, affect the apical localization of Rab11a through Crb complex.

We make the conclusion from both the co-IP and immunohistochemistry dataset. The immunohistochemistry dataset including the localization of Crb2a, Rab11, aPKC, and F-actin in WT, the localization of Crb2a, aPKC, and F-actin in Rab11a/11ba mutants, the localization of Crb2a, Rab11 and F-actin in aPKCλ mutants, and the localization of Crb2a, Rab11 , aPKC and F-actin in nok mutants.

In aPKC mutants, we frequently observed a heterogeneity for the localization of Crb2a and Rab11 in lens epithelia. Both Crb2a and Rab11 lost their apical localization in most of lens epithelia. However, Rab11a was still colocalized with Crb2a in the lens epithelia in which Crb2a was localized at the apical domains (Fig 6I, arrows). To us, it is another evidence to support our conclusion: Crb complex (through MPP5-Rab11 interaction), but not Par6-aPKC-Cdc42 complex, directly adjust the apical localization of Rab11. We have revised the text to better describe the results and methods (line 299-321, line 509-514).

Second: The way the quantification was done for Figure 6L is difficult to understand. The

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quantification should compare the apical and basal distribution in nok mutants versus wild-type. Moreover, the results of the fig6K pointing to a heterogeneity in the apical localization defect for aPKC should be more discussed in the result and not only shortly mentioned in the legend of the figure. This heterogeneity for aPKC apical localization of between anterior and lateral epithelial cells is also an issue for the quantification figure L made from the data of the figure 6K. How is this heterogeneity taken into account for quantification?
Thanks for the reviewer's comments. In wild-type embryos, all Rab11, Crb2a and aPKC were highly and consistently aggregated at the apical domains, both in anterior lens epithelia (defined as 5 cells at the anterior lens, Fig5G shows the schematic illustration) and lateral epithelia (defined as 4 dorsal lateral and 4 ventral lateral cells. The definition was introduced in Methods/Quantifications, line 508-514). However, we consistently observed a heterogeneity for the apical localization of aPKC in nok mutants between anterior lens epithelia and lateral epithelia. The reason that causes the heterogeneity is largely unknown yet, probably some proteins play redundant functions (for example, Pak1 may replace aPKC to build cellular polarity. Aguilar-Aragon et al, 2018). The heterogeneity between anterior and lateral lens epithelia in nok mutants could be used to analyze the association of the localization among Rab11, Crb2a, aPKC and F-actin. The anterior and lateral epithelia serve as the control to each other. We observed that all of these proteins lost their apical enrichment at the anterior lens epithelia in nok mutants. However, in lateral lens epithelia in nok mutants, both Crb2a and Rab11 concurrently lost their apical enrichment, while aPKC and F-actin still showed apical enrichment (high apical/basal relative intensity, Fig6K). Thus, we believe it is another evidence to suggest the direct association between Crb and Rab11, but not between aPKC and Rab11, and do not need to compare with wild-type lens. We analyzed 10 embryos/20 retinas and performed statistics to analyze the heterogeneity. We have revised the text to better describe the results (line 299-321).

Third: Relationships between the actin cytoskeleton Crumbs and aPKC
On the basis of the results presented, it is very difficult if not impossible to draw conclusions concerning the actin network, Crb and aPKC. Moreover the comparison between what can be an actin network between figures 6 F' and 6G' is very difficult because the scale of the figures is not the same and if one enlarges figure F' it seems that one also finds fibers with the orientation of the fiber in G'. Thus the conclusions concerning the links between the active network, aPKC and Crb should be on my opinion removed from the results. Furthermore, the title of this part of the results needs to be changed as it is not supported by the results.
Thanks for the reviewer's comments and suggestions. We have accordingly remove the Fig6F, 6F', 6G and 6G', and revised the subtitle and text (line 299-321).

Minor points
In the Figure S1 panel E, the scale bar is in mirror image
Thanks for the reviewer's comments. We have revised the image.

In the result section line 191 "In detail, either sporadic over-expression generated by plasmid injection (Fig. 3M) or overall over-expression generated by mRNA injection (Fig. 3O) of Rab11a did not significantly affect the apical enrichment of Nok in WT lens epithelia". I think, the reference to Fig. 3O should be replaced by the reference to figure 3K.
Thanks for the reviewer's comments and sorry for the mistake. We have revised the reference.

Reviewer 2 Advance Summary and Potential Significance to Field:
In this manuscript, the authors reported that zebrafish Stardust homologue Nok interacts with rab11 to promote exocytosis of AJ components, cadherin and Crumbs, leading to the establishment of the apical domain polarity and adherens junction in zebrafish lens epithelium. The authors also found that Nok recruits Par complex, which does not interact with rab11 but assembles cytoskeleton to support the exocytosis of adherens junction components. These data suggest a novel role of rab11 and Nok in the formation of adherens junction of zebrafish lens epithelium.
Thanks for the reviewer's comments and suggestions. Please see below for our point-to-point responses.

Reviewer 2 Comments for the Author:
I have reviewed the revised manuscript. The authors have responded to my suggestion. In accordance with my suggestion (20), the authors have added the description on how EGFP-tagged rab11

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expression is introduced. However, the authors did not mention how to prepare DNA constructs expressing EGFP-tagged rab11a and EGFP-2A-rab11a in Materials and Methods; rather they simply stated that the DNA constructs: Tol2[EF1a:rab11a-EGFP], Tol2[EF1a:rab11a(S25N)-EGFP], Tol2[EF1a:rab11a(Q70L)-EGFP], Tol2[EF1a:rab5(S36N)-EGFP] as well as pCS2[CMV promoter(?): EGFP-2A-rab11a] and pCS2[CMV promoter(?): EGFP-2A-rab11a(S25N)] were used for experiments shown in Figure 3D-F; 3K-P, Fig. S6 and Fig. 4IJ.

My concern is whether Tol2[EF1a:rab11a-EGFP] expresses C-terminal EGFP-tagged rab11a or N-terminal EGFP-tagged rab11a. My understanding is that the C-terminal cysteine containing domain of small G-proteins including rab5 and rab11 is important for membrane anchoring, and that localization of rab5 and rab11 to early and recycling endosome (or TGN) is critical for their functions. Thus, N-terminal EGFP tagged rab proteins are generally used for such an overexpression experiment. Although one reference the authors cited (Chen et al., 1998) used N-terminal

EGFP-tagged rab11, it is obscure whether the authors used N-terminal EGFP-tagged rab11/rab5 in their expression constructs. If the authors used C-terminal EGFP tagged rab11/rab5, interpretation of data on Figure 33D-F; 3K-P, Fig. S6 and Fig. 4IJ is difficult. In general, N-terminal GFP-tagged rab11 and rab5 signals are usually observed as dotted signals corresponding to recycling endosomes and early endosome respectively, both overexpressed rab11-EGFP and rab5-EGFP signals were likely to be more broadly observed in cytoplasmic region of lens cells in Fig. 3D-F; 3M-P. Thus, I wonder if the authors used C-terminal EGFP tagged rab11 and rab5 for these overexpression experiments. Please confirm that N-terminal EGFP-tagged rab11 and rab5 were used, and state DNA construction procedures in detail in Materials and Methods. If the authors used N-terminal EGFP-tagged rab11, the authors must state “Tol2[EF1a: EGFP-rab11a]”.
Thanks for the reviewer's comments and we are sorry for the mistake. We used N-terminal eGFP-tagged Rab11a and Rab5 in this study. The constructs (Tol2[EF1a:rab11a-EGFP], Tol2[EF1a:rab11a(S25N)-EGFP], Tol2[EF1a:rab11a(Q70L)-EGFP], Tol2[EF1a:rab5(S36N)-EGFP]) were generated in Dr. Link’s lab (co-author in this study) and firstly used in the paper ‘Clark et al., 2011, Dev. Dyn. 240, 2452-2465’). The constructs (pCS2[T7: EGFP-2A-rab11a] and pCS2[T7: EGFP-2A-rab11a(S25N)]), which were used to perform the in vitro transcription, were generated in Zou’s lab (corresponding author in this study), by adding 2A peptide between eGFP and Rab11a by overlapping PCR based on the above constructs. We have accordingly revised the name of constructs and provided the information in the Methods (line 415-423).

On the other hand, overexpression data using pCS2[EGFP-2A-rab11a] and pCS2[EGFP-2A-rab11a(S25N)] (Figure 3KL) are acceptable from experimental design point of views. However, Fig. 3KL showed the indication of “rab11a-2A-EGFP” and “rab11a (S25N)-2A-EGFP”, which also causes confusion: I wonder which “EGFP-2A-rab11a” or “rab11a-2A-EGFP” the authors introduced into lens. In addition, Fig. 3 legend (see the line 733) stated that rab11a (S25N)-2A-EGFP was overexpressed in lens (N and O), which is inconsistent with figure panel indication in using rab11a-EGFP and rab11a(S25N)-EGFP. Please check your experiment designs and data of Figure 3 as well as text description in your manuscript.
Thanks for the reviewer's comments and suggestions. The Fig3K and 3L are mRNA injection, and Fig3N and 3O are plasmid injection. Line 773 is a mistake and we are very sorry for that.

If the authors unfortunately used C-terminal EGFP/mCherry-tagged rab11 or rab5, my suggestion is that the authors should delete all the data on Figure 3DEF, 3MNOP; rather the authors should reexamine using N-terminal EGFP-tagged rab11/5 or conduct experiments to show Crb2 protein expression of wild-type lenses injected with mRNA of EGFP-2A-rab11a and EGFP-2A-rab11a (S25N), as well as experiment using EGFP-2A-rab5 (S36N) mRNA. The authors also need to reevaluate reciprocal dependency between Rab11 and Crb complex in retinal neuroepithelia (Fig. S6), and in vitro expression data (Fig. 4IJ, and 4LMOP), using N-terminal EGFP/mCherry tagged rab11 constructs.
Thanks for the reviewer's comments and we are sorry for the mistake. We used N-terminal eGFP-tagged Rab11a and Rab5 in this study. We have accordingly revised the name of constructs and provided the information in the Methods.

Another point that I would like to request is that figure panels should appear in the alphabet order in the main text of the results. For example, as Fig. 1 panels appear in the main text as 1AB->1EF->1CD; Fig.3 as 3DEF->3M-> 3GH; Fig. 6 as 6EFG->6JL-> 6HI-> 6K; Fig. S3 as S3A-D->S3G->S3EF). In addition, three supplementary figures S2, S3, S4 hang on Figure 2, and citation order of these

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supplementary figures is also intermingled across S2-S4 in the text, which makes us difficult to follow the logics of results. Please revise in the text or change panel configuration of Fig. S2-4 as well.
Thanks for the reviewer's comments and suggestions, and we have accordingly adjust the order of the figures.

The third, I would like to request the authors to revise the discussion. There are several mistakes. For example, line 337: Fig. S5A-> Fig. S6AB; line 379: out->our. Some sentence such as line 336-338 need to be revised grammatically.
Thanks for the reviewer's comments and suggestions. We have now carefully revised the discussion and revised the mistakes.

Specific comments
1) Line 110: Does “the intersection foci of cells” mean the vertex point where three neighbor cells meet? Fig. 1A does not show the apical plane of lens epithelium because epidermal to epithelial transition does not occurs at 24 hpf. So, the authors may mention simply that E-cadherin is observed as foci, which are generally associated with plasma membrane, especially enriched at the vertex where three neighbor cells meet.
Thanks for the reviewer's comments and suggestions. We have used “the vertex point where three neighbor cells meet” to replace “the intersection foci of cells”.
2) Line 721-722: Do not insert the explanation sentences on other figure panels in Figure 1 legend. Orientation of lens should be described in each figure legends.
Thanks for the reviewer's comments and suggestions, and we have accordingly revised the figures and figure legends.
3) Line 147: the lateral interfaces between -> the basal and lateral interfaces between
Thanks for the reviewer's comments and we have revised the mistake.
4) Line 175-179: Crb2a signals are not observed in Fig. 3E; it is also difficult to tell that the constitutive active form of rab11-EGFP signals is localized in apical domain of WT lens epithelium from the images of Fig. 3E; it is also difficult to tell that rab11-EGFP signals are localized in the apical domain of WT lens epithelium from the image of Fig. 3M. Thus, please replace these images with more convinced ones. I suggest the authors to show more higher magnification images.
Thanks for the reviewer's comments and suggestions, and we have accordingly replace the Fig 3E and 3M.
5) Line 183: Fig. S4-> Fig. S5
Thanks for the reviewer's comments and we have revised the mistake.
6) Line 193: Fig. 3O -> Fig. 3K
Thanks for the reviewer's comments and we have revised the mistake.
7) Line 296: absence -> absent
Thanks for the reviewer's comments and we have revised the mistake.

Reviewer 3 Advance Summary and Potential Significance to Field:
Hao and colleagues have now added new data and clarifications to the current version of this manuscript. I greatly appreciate the efforts the authors took to discriminate the functions of Rab5 and Rab11 during exocytosis and endocytosis. I also appreciate their effort to perform co-IP using not only mammalian cell extracts and also fish eye extracts.
Reviewer 3 Comments for the Author:
However I have two major problems that remain.
Thanks for the reviewer's comments and suggestions. Please see below for our point-to-point responses.

1. They authors either in their response letter and in the revised manuscript reach “ the conclusion that the localization of Rab11 is associated Crb2a with but not actin; and the localization of actin is associated with aPKC, but not Crb2a. This conclusion is based on the observations: “In aPKCλm567 mutants, the immunostaining signal of aPKC was lost, actin also lost its apical localization; however, both Rab11 and Crb2a signals were co-localized at the apical regions in some epithelia (Fig. 6H and 6I). In nok mutants, the immunostaining signals of both Rab11 and Crb2a were lost in all lens epithelia, while both aPKC and actin were enriched at lens lateral epithelia (Fig. 3C, 6K, and 6L)” This conclusion bothers me because I don't think the data support it. I am afraid that the authors force too much on this conclusion because the co-IP experiments did not show an interaction between Rab11 and aPKC.
Hao et al. wrote “Rab11 and Crb2a signals were co-localized at the apical regions in some epithelia”.

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However, in Figure 6I, the distribution of Rab11 seems to be strongly affected and that of Crb2a very disturbed. Similarly, in Figure 6K, the aPKC and actin stainings are affected in a nok mutant background.

Thanks for the reviewer's comments and suggestions. We make the conclusion from both the co-IP and immunohistochemistry dataset. The immunohistochemistry dataset including the localization of Crb2a, Rab11, aPKC, and F-actin in WT, the localization of Crb2a, aPKC, and F-actin in Rab11a/11ba mutants, the localization of Crb2a, Rab11 and F-actin in aPKCλ mutants, and the localization of Crb2a, Rab11 , aPKC and F-actin in nok mutants. In WT, Rab11 share similar expression and localization pattern in lens cells with Crb2a (Fig3A and 3B), but not with aPKC (Fig6A-6C). In Rab11a/11ba mutants, Crb2a, but not aPKC, lost its apical localization (Fig6H). In aPKCλ and nok mutants, we frequently observed heterogeneity for the localization of these proteins in lens epithelia. The reason that causes the heterogeneity is largely unknown yet, probably some proteins may play redundant functions (for example, Pak1 may replace aPKC to build cellular polarity. Aguilar-Aragon et al, 2018). In aPKCλ mutants, we observed that both Crb2a and Rab11 lost their apical localization in most of epithelia. However, Rab11a was still colocalized with Crb2a in the epithelia in which Crb2a was localized at the apical domains in lens epithelia (Fig6G, arrows). In nok mutants, we frequently observed a heterogeneity for the apical localization of aPKC in nok mutants between anterior lens epithelia and lateral epithelia. The heterogeneity between anterior and lateral lens epithelia in nok mutants could be used to analyze the association of the localization among Rab11, Crb2a, aPKC and F-actin. The anterior and lateral epithelia serve as the control to each other. We found that all of these proteins lost their apical enrichment at the anterior lens epithelia in nok mutants. However, in lateral lens epithelia in nok mutants, both Crb2a and Rab11 concurrently lost their apical enrichment, while aPKC and F-actin still show apical enrichment (high apical/basal relative intensity, Fig6L). We believe that all these datasets consistently support the conclusion that the localization of Rab11 and Crb2a are reciprocally dependent. We have revised the text to better describe the observation and conclusion (line 299-321).

2. Hao and colleagues wrote in the manuscript: “We observed that the apical enrichment of F-actin is closely associated with aPKC localization in lens epithelia, rather than localization of Crb complex (Fig. 6D, 6I, and 6J). Cytoskeletal components were organized perpendicularly to the cell-cell contact surface in WT surface epidermis cells, but were oriented in parallel in aPKCλ mutants (Fig. 6G and 6H).” I don't think the authors have sufficient resolution using confocal microscopy to reach this conclusion. I have even more problems with TEM. In WT context, Hao and colleagues show perpendicular actin fibres relative to the cell-cell contact surface. These fibres are at a certain distance from the cell membrane and the adhesion plaque. In Figure 6F', the size of an adhesion plaques is 3mm. In a mutant context for aPKC, the authors detect actin fibres immediately parallel to the adhesion plaques and do not show anything at the same distance as in the WT situation. However, at the right end of the figure, do we not see parallel actin fibres? Moreover, on Figure 6G', the size of an adhesion plate is 4 mm. Clearly Figures 6F' and 6G’ are not at the same resolution even if the scale bar would tend to make us believe it.

Thanks for the reviewer's comments and suggestions. The confocal microscopy can distinguish the apical zone, lateral zone and basal regions of the lens epithelia and the vertex point where two epithelial cells meet. Thus, as defined in Fig5G, we consistently measured the intensity at the vertex point with the same size both at the apical zone (green circle in Fig5G) and at the basal regions (red circle in Fig5G). Combining the statistics (10 embryos/20 retinas), we believe it is reasonable to measure the relative intensity between apical and basal regions using confocal images. We have revised the text to better describe the methods (line 509-514).

For the TEM, we also observed the size difference of cytoskeleton and the distance difference from the adhesion plaque to the cytoskeleton between WT and aPKCλ mutants. The size difference of cytoskeleton is not because of the imaging processing of the figures, please check below for the unprocessed original images. Another difference between WT and aPKCλ mutants is there are lots of proteins signals between adhesion plaque/cell membrane and the cytoskeleton in WT, however, the signals looks like missing in aPKCλ mutants. Given it has been reported that aPKC modulate the organization of cytoskeleton (Uberall et al., 1999; Betschinger et al., 2003; Harris and Peifer, 2007; Hosono et al., 2015), these differences may suggest some (adapter?) proteins is missing in the cytoskeleton. Considering that we have no detail data to explain these differences, we have now removed Fig 6F, 6F’, 6G, and 6G’.

We have removed unpublished data provided for the referees in confidence.

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In conclusion, Hao and colleagues produced a great amount of work in demonstrating the novel role of Nok/Rab11 complex in the apical polarity establishment. I would not recommend adding the data relative to aPKC/actin as they don't appear robust enough. I would then recommend rewriting the summary accordingly. Under these conditions, it remains to be decided whether this dataset is sufficiently new to justify publication in Development.
Thanks for the reviewer's comments. In this study, we elucidated that Nok and Rab11 form a complex to guide the apical exocytosis of cadherin molecules during adhesion junctions remodeling. In the study, we also observed some interesting phenotypes, such as the heterogeneity for the relationship between Crb-MPP5 complex and Par6-aPKC complex in different lens epithelia, and the cytoskeleton orientation modulated by aPKC. The detail mechanisms remain to be elucidated in future.

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Third decision letter

MS ID#: DEVELOP/2019/184457

MS TITLE: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens

AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, Jiancheng Liang, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and two of the referees are happy with your revisions. However one of the reviewers still has many suggestions for improving the manuscript. All of the suggestions are constructive and most are straightforward to address. However there are a couple of suggestions that may require additional experiments; I am not sure whether you are in the position to yet be able to perform labwork but if you are, please do consider these experiments as they could potentially strengthen your conclusions. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so

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within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

In this paper Yumei Hao and colleagues make use of the zebrafish lens and retina development to address in vivo the coordination between the small GTPase Rab11, adherens junction (AJ) remodeling and polarity proteins. This is an important issue since tissue morphogenesis require an important interconnection between membrane trafficking and cell polarity but the mechanisms involved are still largely unknown.

In this revised version, Yumei Hao and colleagues have substantially improved their manuscript and modified accordingly the figures. The authors provided detailed information to the comments and questions submitted by the reviewers.
The manuscript has improved since the last submission and I would recommend its publication in Development.

Comments for the author

In this revised version, Yumei Hao and colleagues have substantially improved their manuscript and modified accordingly the figures. The authors provided detailed information to the comments and questions submitted by the reviewers.

They have substantially improved their manuscript and modified accordingly the figures.

Reviewer 2

Advance summary and potential significance to field

The manuscript submitted by Hao et al. shows that Nok and Rab11 physically interact and promote their apical localization in zebrafish lens epithelium, which is important to keep a structural integrity of lens epithelial polarity and adherent junction remodeling during development. The authors also investigated the relationship between another apical cell polarity regulator aPKC to rab11 in lens epithelial polarity establishment and AJ formation, and found that aPKC seems to function earlier than rab11 and Nok. The aPKC knockdown causes defects in apical enrichment of F-actin but less affects apical localization of Nok and rab11. On the other hand, apical localization of aPKC does not depend on rab11 and nok. These data are interesting, because rab11 dependent trafficking mechanism plays a critical role in lens epithelial polarity establishment through Nok functions.

Comments for the author

I have reviewed a revised manuscript of Hao et al. The manuscript is quite improved by responding to my previous suggestions; however, there are still mistakes and incorrect wording, which need to be revised or corrected appropriately before publication. In addition, I would like to request a couple of experiments because there is no statistical analysis of co-localization of Nok and Rab11 in Golgi (see suggestion (12)), more quantitative analysis is required for aPKC/actin localization in wild-type and rab11 KD (see suggestion 16) and wild-type positive control is missing (see suggestion 17). Other comments are more stylistic revision. Detailed comments are shown below. I believe that these my suggestions make this paper more valid enough to publish in Development.

(1) The authors mentioned that MPP5a and rab11 orientate the exocytosis of both Crb complex and AJs components to apical domain as one of conclusion in the abstract and the summary paragraph of the Introduction. However, this conclusion is still speculative because it is only supported by a couple of indirect evidence: colocalization of rab11/Crbs with Golgi markers and no

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phenotypes of rab5 KD. The authors did not show the direct imaging data that indicate defects in apical vesicular trafficking in lens epithelial cells of MPP5a or rab11 KD. It is acceptable to mention the possibility that MPP5a and rab11 orientate the exocytosis of both Crb complex and AJs components to apical domain in the discussion, but the authors should tone down the wording "exocytosis" in the abstract and introduction. I suggest the authors to rewrite the sentences below.
Line 27: synergistically guide the exocytosis of cadherin and Crumbs component -> synergistically transport cadherin and Crumbs component to the apical domain
Line 97: orientate the apical vesicle exocytosis of both Crb complex and AJs components -> transport both Crb complex and AJs components to the apical domain
Line 100: stochastic exocytosis of Crb components -> failure in the apical transport of Crb components
(2) Grammatical error: line 116: AJs mainly rearranged into -> AJs were mainly rearranged into
(3) Line 884, Legends of Figure S1 (D): There is no description of box 3 and 4. Please add the explanation.
(4) Line 132 and Line 853: Concurrent with -> Consistent with or Coupling with
(5) Line 890, Legends of Figure S2 (A and B): Crb2a was not expressed started to be expressed in lens epithelia at 28 hpf (B) -> Crb2a started to be expressed in lens epithelia at 28 hpf (B), (delete "was not expressed")
(6) Line 908, Figure S3 (C): Legend mentions that arrowheads show the non-specific staining of the antibody. However, there is no arrowhead in figure panel (C). Furthermore, there is no statement on scale bar. Please revise them.
(7) Legends of Figure S4 (A and B; F, G and H): The authors may call individual lens epithelial cells as "epithelia", but "epithelial cells" are more appropriate, so "epithelia" should be changed to "epithelial cells".
Line 916, Lens epithelia migration -> lens epithelial cell migration; Line 917, The lens epithelia in WT were stable in the surface… -> WT lens epithelial cells were stably positioned in the surface…; Line 918, A number of epithelia in nok mutants -> A number of epithelial cells in nok mutants; Line 919, an invading epithelium -> an invading epithelial cell
(8) Line 163-164: The ingressed epithelia eventually differentiated into lens fiber cells, resulting in an increase of the number of lens fiber cells (Fig. S4F-S4H). This sentence misleads readers. As an alternative possibility, increase in the number of lens fiber cells in nok mutant may be due to failure or delay of denucleation of lens fiber cells, whereas decrease in the number of lens epithelial cells in nok mutants may be due to cell death of ingressed lens epithelial cells. So, I suggest the authors to simply mention the results on Figure S4F-S4H as following.

At 36 hpf, the number of lens epithelial cells is lower in nok mutants than in wild type, whereas the number of lens fiber cells is higher in nok mutants than in wild type. The ingressed epithelial cells may progressively differentiate into lens fiber cells in nok mutants. Alternatively, the ingressed epithelial cells may be eliminated by cell death and the denucleation of lens fiber cells may be delayed in nok mutant.
(9) Line 174: Specify the stages (24 hpf and 36 hpf): Rab11 did not exhibit …in epidermal cells (Fig. 3A), … expressed (Fig. 3B). In contrast,… (Fig. 3C). -> Rab11 did not exhibit …in epidermal cells at 24 hpf (Fig. 3A) , … expressed at 36 hpf (Fig. 3B). In contrast,… at 36 hpf (Fig. 3C).
(10) Line 179: "nok" should be italic.
(11) Line 182: Fig3F -> Fig. 3F
(12) Line 254: The authors mention that 29% of eGFP-rab11a foci and 41% of Nok-mCherry foci were colocalized in Golgi. However, Fig. 4LM and also Fig. 4OP indicate that both eGFP-rab11a and Nok-mCherry signals are very broad but do not display dot-like foci. How did the authors evaluate the data? Although the authors provided experimental procedures on evaluation of colocalization to Golgi and ER in Materials and Method section, I would like to request the authors to add more in detail description of experimental procedures, especially how foci are defined from such high background using what kind of image software was used. Furthermore, because the data on colocalization to Golgi are important to conclude exocytosis involvement, I would like to request the authors to do a statistical analysis. Fig. 5N is not enough. The authors should compare the fraction of Nok/GM310 double positive foci in the total number of Nok foci between normal and rab11 KD condition and confirm that the fraction of Golgi co-localized Nok foci is significantly decreased in the absence of rab11 activity. Similarly, the author should confirm that the fraction of Golgi co-localized rab11 foci is significantly decreased in the absence of nok activity.
(13) Line 226: the current evidence on exocytosis are indirect and weak. So, the subtitle "MPP5a interplays with Rab11 to orientate the vesicle exocytosis" is too strong. The subtitle should be

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tone-down like “MPP5 physically associates with rab11 to promote their reciprocal apical localization”.
(14) Line 269 and line 840: The authors mentioned that the number of apical punctum adherens between lens epithelial cells and lens fiber cells “significantly” reduced in rab11 double KD (Fig. 5K). However, there is no statistical analysis on the number of AJs in Fig. 5K. Please add statistical analysis data.
(15) Line 305: The authors mentioned that both Crb2a and Rab11 lost their apical localization in most of lens epithelia in aPKC-lambda mutants. Please show the percentage of normal apically Crb2a and Rab11-localized lens epithelial cells in the total lens epithelial cells in aPKC mutants at 36 hpf.
(16) Fig. 6H: Is the stage 36 hpf? aPKC signals are weak in rab11 double KD, compared with that of Figure 6C. More quantitative analysis is better, so I would like to ask the authors to show the apical/basal relative intensity of aPKC and actin in wild-type and rab11 double KD, as like Fig. 6J. Dot-plot histogram is useful to show heterogeneity of apical localization of aPKC and actin signals as well.
(17) Fig. 6J: Apical localization of aPKC and actin is less affected in the lateral region of lens epithelium of nok mutants, compared with that of Crb2a, rab11. Please show the same histogram of wild-type, which is a positive control.
(18) Line 313 and 315: nok should be italic.
(19) Line 355: The dysfunction of Crb complex leads to the stochastic orientation of rab11-mdiated vesicle traffic to apical interface …. This sentence is too strong, because there is no evidence to show the stochastic orientation of rab11-mdiated vesicle traffic by imaging data. So, this sentence should be revised as following:
One possibility is that the dysfunction of Crb complex leads to the stochastic orientation of rab11-mdiated vesicle traffic to apical interface ….
(20) Line 387: The interplay between Nok and Rab11 guides the apical exocytosis to synergistically establish the apical polarity as well as …. The conclusion may be tone-down. -> The interplay between Nok and Rab11 synergistically establish the apical polarity as well as …, probably through the regulation of the apical exocytosis.
(21) Figure 5EF: Indication of rab11a-2A-eGFP and rab11a S25N-2A-eGFP should be corrected to eGFP-2A-rab11a and eGFP-2A- rab11a S25N.
(22) Subtitle of Figure 4 may be changed into “Nok interplays with Rab11 to promote membrane association of beta-catenin in human cultured cells”.
(23) Line 821, legend of Fig. 3K: in E-I -> in F-J
(24) Line 822, legend of Fig. 3: (L-N) -> (L-P)
(25) Line 825, legend of Fig. 3: mCherry -> Nok-mCherry
(26) Line 828, legend of Fig. 3: Scale bar size of M and P seems to be one-fourth of scale bar size of L and O. If this is the case, Scale bar size of M and P should be 2.5 micro-m. Please check whether scale bar size is correct.
(27) Line 830, Legend of Fig. 5EF: Please add the explanation on arrows.
(28) Line 850: Subtitle of Figure 6 may be changed into “aPKC organizes cytoskeleton to promote AJ remodeling, but apical localization of aPKC does not require Rab11”, because Figure 6 does not contain the direct evidence of apical exocytosis.
(29) Line 851: display -> displays
(30) Line 853: transferred -> accumulated
(31) Legend of Fig. 6BC: Need explanation on arrows.
(32) Line 859, Legend of Figure 6D: There is no statement on square.
(33) Legend of Fig. 6E: Need explanation of arrow and white arrowhead.
(34) Legend of Fig. 6FGH: Need explanation on arrow.
(35) Legend of Fig. 6I: Make the explanation on arrow consistent (see line 865 and line 869). Need explanation of broken (dotted) arrow.
(36) Line 872: Fig. 6K -> Fig. 6I

Reviewer 3

Advance summary and potential significance to field

In this third version of their manuscript, Hao and colleagues have considerably improved the reading of text and figures. The order of the figures now appears much more logical.

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The authors have also clarified their conclusions without over-interpreting their results. I particularly appreciate that data on the organization of the actin network from TEM either in a wild-type or mutant context for aPKC have been removed. The fact that these data have been removed does not undermine from the most important conclusions of this work.
I also appreciate explanations on the quantifications method (line 509-514).
I believe that the latest revisions sufficiently address all reviewers concerns and the manuscript is ready to move forward.

Comments for the author

In this third version of their manuscript, Hao and colleagues have considerably improved the reading of text and figures. The order of the figures now appears much more logical.
The authors have also clarified their conclusions without over-interpreting their results. I particularly appreciate that data on the organization of the actin network from TEM either in a wild-type or mutant context for aPKC have been removed. The fact that these data have been removed does not undermine from the most important conclusions of this work.
I also appreciate explanations on the quantifications method (line 509-514).
I believe that the latest revisions sufficiently address all reviewers concerns and the manuscript is ready to move forward.

Third revision

Author response to reviewers' comments

Dear Editors and reviewers,

We thank the editors and reviewers for their efforts in evaluating our manuscript again and their constructive comments. In this version, we have revised figure 5K and 6K, added new figure 6I and figure S8 according to the reviewers' comments and suggestions. Considering the figure 4 is already big, we organized the requested new data into figure S8. We also revised the text (marked with blue color in the manuscript). In addition, Ziqi Kou made contributions for the paper in the revision, thus we added his name in the author list. We believe that all concerns/questions raised by the reviewers have been addressed, and the revised manuscript is significantly improved.

Reviewer 1 Advance summary and potential significance to field
In this paper Yumei Hao and colleagues make use of the zebrafish lens and retina development to address in vivo the coordination between the small GTPase Rab11, adherens junction (AJ) remodeling and polarity proteins. This is an important issue since tissue morphogenesis require an important interconnection between membrane trafficking and cell polarity but the mechanisms involved are still largely unknown.

In this revised version, Yumei Hao and colleagues have substantially improved their manuscript and modified accordingly the figures. The authors provided detailed information to the comments and questions submitted by the reviewers. The manuscript has improved since the last submission and I would recommend its publication in Development.

Reviewer 1 Comments for the author
In this revised version, Yumei Hao and colleagues have substantially improved their manuscript and modified accordingly the figures. The authors provided detailed information to the comments and questions submitted by the reviewers.

They have substantially improved their manuscript and modified accordingly the figures.

Thanks for the reviewer's positive comments.

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Reviewer 2 Advance summary and potential significance to field
The manuscript submitted by Hao et al. shows that Nok and Rab11 physically interact and promote their apical localization in zebrafish lens epithelium, which is important to keep a structural integrity of lens epithelial polarity and adherent junction remodeling during development. The authors also investigated the relationship between another apical cell polarity regulator aPKC to rab11 in lens epithelial polarity establishment and AJ formation, and found that aPKC seems to function earlier than rab11 and Nok. The aPKC knockdown causes defects in apical enrichment of F-actin but less affects apical localization of Nok and rab11. On the other hand, apical localization of aPKC does not depend on rab11 and nok. These data are interesting, because rab11 dependent trafficking mechanism plays a critical role in lens epithelial polarity establishment through Nok functions.
Thanks for the reviewer's very careful comments and suggestions.

Reviewer 2 Comments for the author
I have reviewed a revised manuscript of Hao et al. The manuscript is quite improved by responding to my previous suggestions; however, there are still mistakes and incorrect wording, which need to be revised or corrected appropriately before publication. In addition, I would like to request a couple of experiments because there is no statistical analysis of co-localization of Nok and Rab11 in Golgi (see suggestion (12)), more quantitative analysis is required for aPKC/actin localization in wild-type and rab11 KD (see suggestion 16) and wild-type positive control is missing (see suggestion 17). Other comments are more stylistic revision. Detailed comments are shown below. I believe that these my suggestions make this paper more valid enough to publish in Development.

(1)The authors mentioned that MPP5a and rab11 orientate the exocytosis of both Crb complex and AJs components to apical domain as one of conclusion in the abstract and the summary paragraph of the Introduction. However, this conclusion is still speculative because it is only supported by a couple of indirect evidence: colocalization of rab11/Crbs with Golgi markers and no phenotypes of rab5 KD. The authors did not show the direct imaging data that indicate defects in apical vesicular trafficking in lens epithelial cells of MPP5a or rab11 KD. It is acceptable to mention the possibility that MPP5a and rab11 orientate the exocytosis of both Crb complex and AJs components to apical domain in the discussion, but the authors should tone down the wording “exocytosis” in the abstract and introduction. I suggest the authors to rewrite the sentences below.
Line 27: synergistically guide the exocytosis of cadherin and Crumbs component -> synergistically transport cadherin and Crumbs component to the apical domain
Line 97: orientate the apical vesicle exocytosis of both Crb complex and AJs components -> transport both Crb complex and AJs components to the apical domain
Line 100: stochastic exocytosis of Crb components -> failure in the apical transport of Crb components
Thanks for the reviewer's suggestions and we have revised the text.

(2)Grammatical error: line 116: AJs mainly rearranged into -> AJs were mainly rearranged into
Thanks for the reviewer's suggestions and we have revised the mistake.

(3)Line 884, Legends of Figure S1 (D): There is no description of box 3 and 4. Please add the explanation.
Thanks for the reviewer's comments and we have revised the text.

(4)Line 132 and Line 853: Concurrent with -> Consistent with or Coupling with
Thanks for the reviewer's suggestions and we have revised the text.

(5)Line 890, Legends of Figure S2 (A and B): Crb2a was not expressed started to be expressed in lens epithelia at 28 hpf (B) -> Crb2a started to be expressed in lens epithelia at 28 hpf (B), (delete “was not expressed”)
Thanks for the reviewer's suggestions and we have revised the text.

(6)Line 908, Figure S3 (C): Legend mentions that arrowheads show the non-specific staining of the antibody. However, there is no arrowhead in figure panel (C). Furthermore, there is no statement on scale bar. Please revise them.
Thanks for the reviewer's comments and we have revised the text.

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(7)Legends of Figure S4 (A and B; F, G and H): The authors may call individual lens epithelial cells as "epithelia", but "epithelial cells" are more appropriate, so "epithelia" should be changed to "epithelial cells".
Line 916, Lens epithelia migration -> lens epithelial cell migration; Line 917, The lens epithelia in WT were stable in the surface… -> WT lens epithelial cells were stably positioned in the surface…; Line 918, A number of epithelia in nok mutants -> A number of epithelial cells in nok mutants; Line 919, an invading epithelium -> an invading epithelial cell
Thanks for the reviewer's suggestions and we have revised the text.

(8)Line 163-164: The ingressed epithelia eventually differentiated into lens fiber cells, resulting in an increase of the number of lens fiber cells (Fig. S4F-S4H). This sentence misleads readers. As an alternative possibility, increase in the number of lens fiber cells in nok mutant may be due to failure or delay of denucleation of lens fiber cells, whereas decrease in the number of lens epithelial cells in nok mutants may be due to cell death of ingressed lens epithelial cells. So, I suggest the authors to simply mention the results on Figure S4F-S4H as following.

At 36 hpf, the number of lens epithelial cells is lower in nok mutants than in wild type, whereas the number of lens fiber cells is higher in nok mutants than in wild type. The ingressed epithelial cells may progressively differentiate into lens fiber cells in nok mutants. Alternatively, the ingressed epithelial cells may be eliminated by cell death and the denucleation of lens fiber cells may be delayed in nok mutant.
Thanks for the reviewer's suggestions and we have revised the text.

(9)Line 174: Specify the stages (24 hpf and 36 hpf): Rab11 did not exhibit …in epidermal cells (Fig. 3A), … expressed (Fig. 3B). In contrast,… (Fig. 3C). -> Rab11 did not exhibit …in epidermal cells at 24 hpf (Fig. 3A) , … expressed at 36 hpf (Fig. 3B). In contrast,… at 36 hpf (Fig. 3C).
Thanks for the reviewer's suggestions and we have revised the text.

(10)Line 179: "nok" should be italic.
Thanks for the reviewer's suggestions and we have revised the text.

(11)Line 182: Fig3F -> Fig. 3F
Thanks for the reviewer's suggestions and we have revised the text.

(12)Line 254: The authors mention that 29% of eGFP-rab11a foci and 41% of Nok-mCherry foci were colocalized in Golgi. However, Fig. 4LM and also Fig. 4OP indicate that both eGFP-rab11a and Nok-mCherry signals are very broad but do not display dot-like foci. How did the authors evaluate the data? Although the authors provided experimental procedures on evaluation of colocalization to Golgi and ER in Materials and Method section, I would like to request the authors to add more in detail description of experimental procedures, especially how foci are defined from such high background using what kind of image software was used. Furthermore, because the data on colocalization to Golgi are important to conclude exocytosis involvement, I would like to request the authors to do a statistical analysis. Fig. 5N is not enough. The authors should compare the fraction of Nok/GM310 double positive foci in the total number of Nok foci between normal and rab11 KD condition and confirm that the fraction of Golgi co-localized Nok foci is significantly decreased in the absence of rab11 activity. Similarly, the author should confirm that the fraction of Golgi co-localized rab11 foci is significantly decreased in the absence of nok activity.
Thanks for the reviewer's comments and suggestions. Now we have added the requested new data. We observed that eGFP-Rab11a, Nok-mCherry or eGFP-Rab11a S25N were spread in the whole cell without aggregation, which is different from the aggregation of eGFP-Rab11a/Nok-mCherry in Golgi, when these proteins were individually expressed in MDCK cells (Fig. S8A-S8D). Also, the co-expression of eGFP-Rab11a S25N /Nok-mCherry did not show the aggregation in Golgi. We have also added more information in the methods to describe the procedures.

(13)Line 226: the current evidence on exocytosis are indirect and weak. So, the subtitle "MPP5a interplays with Rab11 to orientate the vesicle exocytosis" is too strong. The subtitle should be tone-down like "MPP5 physically associates with rab11 to promote their reciprocal apical localization".
Thanks for the reviewer's comments and suggestions. We have revised the text.

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(14)Line 269 and line 840: The authors mentioned that the number of apical punctum adherens between lens epithelial cells and lens fiber cells “significantly” reduced in rab11 double KD (Fig. 5K). However, there is no statistical analysis on the number of AJs in Fig. 5K. Please add statistical analysis data.
Thanks for the reviewer's comments and suggestions. The data for AJ_EFs (apical punctum adherens between lens epithelial cells and lens fiber cells) in rab11a/ba dKO is shown in group 4 column 4. Now we have added statistical P data.

(15)Line 305: The authors mentioned that both Crb2a and Rab11 lost their apical localization in most of lens epithelia in aPKC-lambda mutants. Please show the percentage of normal apically Crb2a and Rab11-localized lens epithelial cells in the total lens epithelial cells in aPKC mutants at 36 hpf.
Thanks for the reviewer's suggestions and we have now added the percentage data in the text.

(16)Fig. 6H: Is the stage 36 hpf? aPKC signals are weak in rab11 double KD, compared with that of Figure 6C. More quantitative analysis is better, so I would like to ask the authors to show the apical/basal relative intensity of aPKC and actin in wild-type and rab11 double KD, as like Fig. 6J. Dot-plot histogram is useful to show heterogeneity of apical localization of aPKC and actin signals as well.
Thanks for the reviewer's comments and suggestions. We have added stage in the figure legends (36 hpf) and the statistical data.

(17)Fig. 6J: Apical localization of aPKC and actin is less affected in the lateral region of lens epithelium of nok mutants, compared with that of Crb2a, rab11. Please show the same histogram of wild-type, which is a positive control.
Thanks for the reviewer's comments and suggestions. We have added the WT data.

(18)Line 313 and 315: nok should be italic.
Thanks for the reviewer's comments and we have revised the text.

(19)Line 355: The dysfunction of Crb complex leads to the stochastic orientation of rab11-mdiated vesicle traffic to apical interface …. This sentence is too strong, because there is no evidence to show the stochastic orientation of rab11-mdiated vesicle traffic by imaging data. So, this sentence should be revised as following: One possibility is that the dysfunction of Crb complex leads to the stochastic orientation of rab11-mdiated vesicle traffic to apical interface ….
Thanks for the reviewer's suggestions and we have revised the text.

(20)Line 387: The interplay between Nok and Rab11 guides the apical exocytosis to synergistically establish the apical polarity as well as …. The conclusion may be tone-down. -> The interplay between Nok and Rab11 synergistically establish the apical polarity as well as …, probably through the regulation of the apical exocytosis.
Thanks for the reviewer's comments and we have revised the text.

(21)Figure 5EF: Indication of rab11a-2A-eGFP and rab11a S25N-2A-eGFP should be corrected to eGFP-2A-rab11a and eGFP-2A- rab11a S25N.
Thanks for the reviewer's comments and we have revised the text.

(22)Subtitle of Figure 4 may be changed into “Nok interplays with Rab11 to promote membrane association of beta-catenin in human cultured cells”.
Thanks for the reviewer's comments and we have revised the text.

(23)Line 821, legend of Fig. 3K: in E-I -> in F-J
Thanks for the reviewer's comments and we have revised the text.

(24)Line 822, legend of Fig. 3: (L-N) -> (L-P)
Thanks for the reviewer's comments and we have revised the text.

(25)Line 825, legend of Fig. 3: mCherry -> Nok-mCherry
Thanks for the reviewer's comments and we have revised the text.

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(26)Line 828, legend of Fig. 3: Scale bar size of M and P seems to be one-fourth of scale bar size of L and O. If this is the case, Scale bar size of M and P should be 2.5 micro-m. Please check whether scale bar size is correct.
Thanks for the reviewer's comments and we have revised the text.

(27)Line 830, Legend of Fig. 5EF: Please add the explanation on arrows.
Thanks for the reviewer's comments and we have revised the text.

(28)Line 850: Subtitle of Figure 6 may be changed into “aPKC organizes cytoskeleton to promote AJ remodeling, but apical localization of aPKC does not require Rab11”, because Figure 6 does not contain the direct evidence of apical exocytosis.
Thanks for the reviewer's comments and we have revised the text.

(29)Line 851: display -> displays
Thanks for the reviewer's comments and we have revised the text.

(30)Line 853: transferred -> accumulated
Thanks for the reviewer's comments and we have revised the text.

(31)Legend of Fig. 6BC: Need explanation on arrows.
Thanks for the reviewer's comments and we have revised the text.

(32)Line 859, Legend of Figure 6D: There is no statement on square.
Thanks for the reviewer's comments and we have revised the text.

(33)Legend of Fig. 6E: Need explanation of arrow and white arrowhead.
Thanks for the reviewer's comments and we have revised the text.

(34)Legend of Fig. 6FGH: Need explanation on arrow.
Thanks for the reviewer's comments and we have revised the text.

(35)Legend of Fig. 6I: Make the explanation on arrow consistent (see line 865 and line 869). Need explanation of broken (dotted) arrow.
Thanks for the reviewer's comments and we have revised the text.

(36)Line 872: Fig. 6K -> Fig. 6I
Thanks for the reviewer's comments and we have revised the text.

Reviewer 3 Advance summary and potential significance to field
In this third version of their manuscript, Hao and colleagues have considerably improved the reading of text and figures. The order of the figures now appears much more logical. The authors have also clarified their conclusions without over-interpreting their results. I particularly appreciate that data on the organization of the actin network from TEM either in a wild-type or mutant context for aPKC have been removed. The fact that these data have been removed does not undermine from the most important conclusions of this work. I also appreciate explanations on the quantifications method (line 509-514). I believe that the latest revisions sufficiently address all reviewers concerns and the manuscript is ready to move forward.

Reviewer 3 Comments for the author
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Thanks for the reviewer's positive comments.

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Fourth decision letter

MS ID#: DEVELOP/2019/184457

MS TITLE: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens

AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, ziqi kou, Jiancheng Liang, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou

I sent your manuscript back to one of the referees and there are just a few minor issues for you to address before we proceed to publication. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

Reviewer 2

Advance summary and potential significance to field

The authors report the rigid evidence that an apical regulator Nok cooperates with rab11 to promote the establishment of apical domain of lens epithelium in zebrafish, suggesting an unexpected role of rab11 in apical polarization and AJ formation in lens epithelium.

Comments for the author

I think that the authors fully respond to my suggestion. However, there are several errors below. These errors should be corrected before the paper is accepted. If the authors correct them, there is no need for another round to review.

1. line 165, 166, 168: "nok" should be italic.

2. line 319: Fig. 6F and Fig. 6G => Fig. 6G
3. line 321: Fig. 3J, 3K, and 6H => Fig. 3J, 3K, 6H and 6I
4. line 322: Fig. 6H => Fig. 6H and 6I
5. line 327: Fig. 6I and 6J => Fig. 6J and 6K
6. line 341: Fig. 6F-6J => Fig. 6F-6H, and 6J

7. line 820: beta-catenin: "beta" should be "symbol font"

8. Fig. 6 legend: explain arrowhead and broken arrow in C.
9. line 895: the authors mentioned that aPKC and actin, but not Crb2a and Rab11, displayed apical enrichment in rab11 dKO mutants, but actin enrichment is significant reduced in rab11 dKO mutants (**p<0.01). So, this description should be revised as below.
aPKC displayed normal apical enrichment in rab11 dKO mutants; however, apical enrichment of actin, Crb2a and Rab11 is significantly decreased although actin reduction is mild.
10. Figure S8 legend, line 96: eGFP-Rab11a (A), Nok-mCherry (B),..... => Nok-mCherry (A), eGFP-Rab11a (B),
.....

Fourth revision

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AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, Ziqi Kou Jiancheng Liang, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou

Dear reviewer,

We thank the reviewers for their efforts in evaluating our manuscript again and their constructive and detailed comments. In this version, we have carefully revised our manuscript and corrected the text errors mentioned by the reviewer 2 and similar errors (marked by blue). We believe that all concerns/questions raised by the reviewers have been addressed, and the revised manuscript is significantly improved.

Reviewer 2 Advance summary and potential significance to field...
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Fifth decision letter

MS ID#: DEVELOP/2019/184457

MS TITLE: Interplay of MPP5a with Rab11 synergistically builds epithelial apical polarity and zonula adherens

AUTHORS: Yumei Hao, Yao Zhou, Yinhui Yu, Mingjie Zheng, Kechao Weng, ziqi kou, Jiancheng Liang, Qian Zhang, Xiajing Tang, Pinglong Xu, Brian Link, Ke Yao, and Jian Zou
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

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