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# Kif3a interacts with Dynactin subunit p150Glued to organize centriole subdistal appendages

Andrew Kodani, Maria Salomé Sirerol-Piquer, Allen Seol, Jose Manuel Garcia-Verdugo and Jeremy F. Reiter

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**Review timeline:**

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## Transaction Report:

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

1st Editorial Decision 26 July 2012

Thank you for the submission of your manuscript to The EMBO Journal. I apologize for the delay in responding, but I have only now received the comments from the two referees that were asked to evaluate your study, which are copied below. I am afraid to say that the evaluation of your manuscript is not a positive one.

As you will see from the enclosed reports, both referees acknowledge the importance of your work, particularly given that centriole appendage/basal feet formation is not well understood. However, the referees also consider that your study is rather preliminary and that your manuscript would not be sufficiently developed at this stage to be considered for publication at the The EMBO Journal.

Considering the nature of these evaluations and the fact that The EMBO Journal can only invite revision of papers that receive strong support from the referees, I am afraid that calling for a revised version of your manuscript at this stage is not justified and therefore we cannot offer to publish it.

I am sorry that I have to disappoint you this time. I hope, however, that the referee comments will be helpful in your continued work in this area and I thank you once more for the opportunity to consider your manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1 (General Remarks):

Disruption of the Kinesin-II motor heterodimeric subunits Kif3a or Kif3b is known to block mammalian ciliogenesis as this motor is required for anterograde IFT transport.
In the present work from the Reiter group, Kif3a is proposed to participate also in the transformation of the subdistal appendages of the basal body into a morphologically distinct structure, the basal foot, upon which cytoplasmic microtubules are anchored .
The main results are the following :

- Association with the centrosome is demonstrated by IF investigation and biochemical analysis of centrosome-enriched fractions.
- A series of experiments tend to support a role of Kif3a in maintaining centriole engagement - or centrosome cohesion (see below) - and in organizing subdistal appendages independently of its function in IFT.
- In particular, Kif3a seems to control the localization of p150Glued to centrosomes, and the loss of p150Glued by siRNA alters centriole cohesion, providing a possible mechanism for the effet of Kif3a on the centrosome.
- p150Glued is convincingly shown by a biochemical approach, to interact with the carboxy-terminal region of Kif3a.
- As expected from the interaction with p150Glued, which is known to be required for microtubule anchoring at the mother centriole, Kif3a is shown to participate in the anchoring of centrosomal microtubules in wild-type and Kif3a-/- MEFs.

Major concerns:

- The Kif3a staining in figure 1 shows that Kif3a seems associated with the daughter centriole and not the mother. In all cases Kif3a localizes to the centriole that is less intensely labelled with centrin antibody (figure 1B and C). There is also very little co-localization between Kif3a and ninein or p150 (figure 1d). This is somehow in contradiction with the experiments presented in figure 4 and 5. EM localization is mandatory to clarify the localisation of the focus of Kif3a and to support the model.

- The role of Kif3a in maintaining ´ centriole engagement ª (page 7) is not supported by the data presented. What is observed in my opinion is the splitting of centrioles already disengaged (fig 2a c). The authors must clarify between centrosome cohesion and centriole disengagement, which are different mechanisms under specific controls.

- Some data are not that novel
In Nigg's paper on Cep164 (Graser at al 2007) it is shown that Cep170 is dependent upon ninein for its localization to the centrosome (figure 4b).

In Nigg's paper on the phosphorylation of Nlp by PLK1 (Casenghi et al 2005) it is shown that p150 interacts with the N-terminus of ninein (figures 2c and 3a) and that the transport of ninein to the centrosome is dynein-dependent (figure 5).

- In the abstract the basal foot in Kif3a-/- is supposed to be disorganized whereas in the Results section it is lacking (page 11), which is slightly disturbing. As a matter of fact, the EM does not support the model in my opinion. In all of the EM images presented (control, Kif3a-/-, IFT88-/-) a single basal foot is visible. In the serial sections presented in fig 6b, which are oblique with respect to the basal body longitudinal axis, the tip of the basal foot is probably present in the top section, thus suggesting that the basal foot is normal. Moreover, I think there are even MTs attached to the basal foot of the Kif3a-/- mother centriole. How many serial sections of basal feet have been observed altogether ?

In conclusion, this paper appears more as a preliminary contribution on a potentially novel and important role of Kinesin II during ciliogenesis. The claim of the title and the two major conclusions of this work, namely that Kif3a specifically associates with the mother centriole and organizes subdistal appendages, are not unambiguously supported by the data in my opinion. I thus cannot support publication of this paper in EMBO J in its present form

Minor :
Page 7, bottom : distal rather than subdistal appendages are mentionned.
Scale bars should be indicated in all IF figures.

Referee #2 (General Remarks):

In this manuscript Kodani and colleagues study the role of the Kif3a kinesin in the organization of the centriolar subdistal appendages. Using mouse embryonic fibroblasts derived from wild-type and Kif3a -/- animals the authors show that Kif3a specifically associates with the mother centrioles. The authors further show that Kif3a is required to maintain centriole cohesion and the organization of subdistal appendages. Indeed they find that Kif3a is required for the localization of p150Glued, Ninein and CEP170 but not ODF2 to these appendages. IFT88 does not appear to be required for the organization of subdistal appendages and the maintenance of centriole cohesion suggesting that Kif3a role in these processes is likely independent of IFT88's role in IFT. The authors go on to confirm previous data on the requirement of p150Glued for centriole cohesion and further show that it is also required for CEP170 and Ninein localization. They also confirm the previously observed interaction between p150Glued and Kif3a in Xenopus and show that this interaction is occurring through the C-terminal cargo-binding region of Kif3a. Using MT anchoring assays, the authors establish a role for Kif3a in MT anchoring and show that it is required for the formation of basal feet. Overall this is a well performed study with data of very high quality. The strength of this study is the elegant use of MEFs and KO animals to study a poorly understood aspect of centriole biogenesis, more particularly appendage formation. The major weakness of this paper concerns the confirmatory aspects of some of the observations (e.g. p150/Kif3a interaction, Kif3a localization to centrioles) and the minimal amount of new molecular insights provided that would further illuminate the precise role of Kif3a in the regulation of subdistal appendage formation. The authors should consider the following points:

- To provide more mechanistic insight on the potential role of Kif3a in the biogenesis of centriole appendages, the authors should perform structure function analysis in wild-type and Kif3a -/- MEFs to look at the ability of various Kif3a truncation mutants to rescue loos of centriole cohesion, cilia formation and basal feet assembly. The ability to restore localization of other components although suggestive of rescue needs to be further substantiated. It would be particular informative if the authors could investigate the role of the motor domain, through point mutations affecting motor activity instead of inferring this from large deletion mutants, in these processes. This would allow them to determine if motor activity is actually required for these processes.

- In microtubule organization experiment, the authors should consider the role of Ninein in MT anchoring in the interpretation of their results. Having shown that Kif3a and p150 are both required for Ninein localization, the fact that less microtubules were anchored at the centrosomes in Kif3a mutants might be a consequence of Ninein mislocalization rather than a direct role for kif3a in microtubule organization and anchoring.

- The authors should carefully analyze the relative levels Ninein, p150Glued and Kif3a upon RNAi of each of these proteins and in wild type cells and in the Kif3a -/- MEFs. This could rule-out potential indirect effects of protein stability when one of the other proteins is missing and is necessary to make statements on the ability of different proteins to localize in absence of each other.

- The author analyze in detail the distribution of Kif3a on centriole and show that its distribution is different than other subdistal appendage proteins, including p150 with which it interacts. The authors should explain what exactly this means and how it fits with their interaction data.

- Along these lines, it is curious that the dimmer of the two Centrin dots are the ones that co-label

with Kif3a. It is generally accepted that the brighter Centrin structure is the mother centriole so this data appears to contradict what is shown in Figure 1D using mother specific markers. The authors should clarify this.

- The distribution of Kif3a on mitotic centrioles is intriguing because it is no longer punctate. The authors should comment on whether or not this is consistent with Kif3a being a subdistal appendage marker.

- The authors should cite previous work on IFT88 mutants in Chlamydomonas and humans that showed that basal bodies are anchored at the plasma membrane in absence if IFT88 as it is related to their observation that IFT88 mutants still have basal feet.

Additional Author Correspondence 27 July 2012

Thank you for overseeing our manuscript and for sending us the reviews. We were heartened to learn that a reviewer described the work as "a well performed study with data of very high quality," and an "elegant use of MEFs and KO animals to study a poorly understood aspect of centriolebiogenesis." Because of the concerns raised, I understand your decision to return the manuscript to us.

Andy and I have spent the last two days discussing the comments made by the reviewers. Fortunately, we have data already in hand thataddress many of the reviewers' points, but were not included in the submitted manuscript because of space concerns . We will be able to address the other comments in a short amount of time (detailed below). Perhaps more importantly, though, we wanted to describe what we see as the significance of this study.

Somewhat paradoxically, the two main points of this paper have to do with an important structure about which almost nothing is known and an important protein about which much is known. The important structure is the basal foot, an appendage of the mother centriole described decades ago. Only one protein is known to be required for basal foot formation, Odf2 (Kunimoto et al. Cell 2012). Thus, the identification of Kif3a as the second protein required to form a basal foot is a major advance for understanding how this structure is created.

Kif3a itself has been intensively studied. However, most within the field think of Kif3a as being specifically required for ciliogenesis. Indeed, deletion of Kif3a and other Kinesin-II components is used by many vertebrate biologists as a way of disrupting ciliogenesis (e.g., Han et al. Nature Neurosci 2008, Koyama et al. Development 2007, Lin et al. PNAS 2003, etc). Our study shows that, in addition to participating in ciliogenesis, Kif3a has aseparate, unanticipated role in basal foot formation. This additional role for Kif3a expands our understanding of Kinesin-II functions, and provides important understanding of how Kif3a mutations affect cell biology apart from ciliogenesis.

The only concern brought up by both reviewers regards whether Kif3a does indeed associate specifically with the mother centriole, as Figure 1 appears to show Kif3a localizing to the centrioles with less Centrin staining. The dimmer Centrin staining results from imaging on aninverted microscope with a single plane of focus. The data included in Fig 1d shows that Kif3a colocalizes with the mother centriole (as marked by Ninein and p150Glued) and not with the daughter centriole (as marked by Centrobin). We have now generated higher resolution images usingdeconvolution microscopy that unambiguously demonstrate that Kif3a localizes specifically to the mother centriole. We are also performing immunoelecton microscopy to confirm that Kif3a localizes to the mother centriole.

In addition, we will be able to address the remaining more minor concerns in a short amount of time (detailed below). Given that we are able to include additional data that address all of the major concerns,would you consider allowing for submission of a revised version of our manuscript? In case it would be helpful in your deliberations, I've added our comments below describing how we would address the additional concerns of the reviewers. Also, I would be happy to provide any additional information you might want, Dr. del Alamo. Please write back or call me any time if I can

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answer any questions or discuss the paper.

Additional points:
Reviewer 1's second point is that we misused the term "centriole engagement" on page 7. I appreciate the correction, and we will fix this mistake.

Reviewer 1, point 3: We will include references to Erich Nigg's work on Cep164 and Dynactin. We were not trying to show novel roles for Cep164 or Dynactin in centrosome organization, but showing that Kif3a recapitulates these phenotypes.

Reviewer 1, point 4: We have analyzed a dozen mother centrioles of Kif3a mutant cells using serial serial section transmission electron microscopy, and none have a basal body. We would emphasize this quantitation and include our additional TEM as a supplemental figure, if a resubmission were to be permitted.

Reviewer 1, minor point: We will be sure to include scale bars on all panels, and will clarify the distal versus subdistal appendage reference onpage 7.

Reviewer 2, point 1: We will extend the structure function analysis of Kif3a beyond the deletion analysis already included by creating point mutations in the motor domain. As pointed out by the reviewer, this will be an alternative test of the involvement of the motor domain in centriolar localization, centriole cohesion, and subdistal appendage formation.

Reviewer 2, point 2: The reviewer suggests that defects in microtubule nucleation in Kif3a mutant cells may be due to the mislocalization of Ninein. We will test this hypothesis by knocking down Ninein and ascertaining whether loss of Ninein is sufficient to disrupt microtubule anchoring.

Reviewer 2, point 3: We will carefully quantify Ninein, p150Glued and Kif3a in all our experimental conditions, as requested.

Reviewer 2, point 4: We will comment more fully on the meaning of minimal colocalization of p150Glued and Kif3a. Like Odf2, Kif3a is required for p150Glued localization to subdistalappendages, but shows only partial colocalization. Thus, centriolar Odf2 and Kif3a help create the foundation on which p150Glued can stably associate with the centriole, but are unlikely to be the interacting partners that stabilize p150Glued at thecentriole themselves.

Reviewer 2, point 6: We will comment on the centrosomal localization of Kif3a during mitosis, as requested.

Reviewer 2, point 7: We will be sure to cite previous work on Chlamydomonas and human Ift88 mutants.

Additional Editorial Correspondence 30 July 2012

Thank you for your e-mail and your responses to the comments of the referees.

As mentioned in my decision letter, both referees consider your manuscript of potential interest. With this in mind, if you can provide experimental evidence that significantly deepens the mechanistic aspect of your manuscript and addresses the concerns of the referees - as your e-mail suggests - I would be glad to reconsider a new manuscript in the near future. To be completely clear, however, I would like to stress that a new manuscript will be treated as a new submission rather than a revision and, although we will try to contact the same referees, it will be re-evaluated in terms of novelty with respect to the published literature at the time of submission.

Thank you very much again for your interest in The EMBO Journal. I look forward to seeing the new version of your study.

Yours sincerely,

Editor

The EMBO Journal

Resubmission 19 November 2012

*Dear Reviewers,*

*We are deeply appreciative for your many helpful comments. Your extensive suggestions have led to quite a number of additional experiments that have significantly strengthened the manuscript. We were encouraged to learn that reviewer 2 wrote that "this is a well performed study with data of very high quality," and that we made "elegant use of MEFs and KO animals to study a poorly understood aspect of centriole biogenesis, more particularly appendage formation." We have spent a substantial amount of time generating additional data and making revisions that address your concerns and questions. We discuss how we have attended to your comments point by point, below, in bold.*

*Reviewer 1:*
*The Kif3a staining in figure 1 shows that Kif3a seems associated with the daughter centriole and not the mother. In all cases Kif3a localizes to the centriole that is less intensely labelled with centrin antibody (figure 1B and C). There is also very little co-localization between Kif3a and ninein or p150 (figure 1d). This is somehow in contradiction with the experiments presented in figure 4 and 5. EM localization is mandatory to clarify the localisation of the focus of Kif3a and to support the model.*

The reviewer correctly pointed out that Kif3a appeared to localize to the centriole with dimmer Centrin staining in some images. We restained and reimaged cells stained with antibodies to Kif3a and Centrin, and found that Kif3a is associated with the centriole with the slightly brighter Centrin staining, presumed to be the mother centriole. Previous images showing Kif3a at Centrin showed localization to the dimmer spot due to an artefact of imaging using an inverted microscope. These new data are included in the new version of the manuscript, and are in agreement with Figure 1d showing that Kif3a colocalizes with the mother centriole specific markers Ninein and $p150^{Glued}$, but not with the daughter specific marker Centrobin. We agree that careful investigation using immuno-EM would clarify the precise localization of Kif3a, but our antibody to Kif3a was not compatible with EM fixation conditions. We respectfully submit that advanced light microscopy shows centriolar localization of both endogenous and tagged versions of Kif3a. We will continue to refine our approaches to determining the precise localization of Kif3a and will include these findings in a future publication.

*The role of Kif3a in maintaining « centriole engagement » (page 7) is not supported by the data presented. What is observed in my opinion is the splitting of centrioles already disengaged (fig 2a c). The authors must clarify between centrosome cohesion and centriole disengagement, which are different mechanisms under specific controls.*

We appreciate the reviewer's point that centriole cohesion and disengagement are distinct events under different regulation. We have corrected our misreferences to centriole engagement.

*In Nigg's paper on Cep164 (Graser at al 2007) it is shown that Cep170 is dependent upon ninein for its localization to the centrosome (figure 4b).*

*In Nigg's paper on the phosphorylation of Nlp by PLK1 (Casenghi et al 2005) it is shown that p150 interacts with the N-terminus of ninein (figures 2c and 3a) and that the transport of ninein to the centrosome is dynein-dependent (figure 5).*

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We appreciate the reviewer correctly pointed out that Cep170 depends upon Ninein for its localization to the centrosome and that the transport of Ninein to the centrosome is dynein-dependent. We have made reference to these previous findings (Page 6, paragraph 2) and (Page 8, paragraph 2).

*In the abstract the basal foot in Kif3a-/- is supposed to be disorganized whereas in the Results section it is lacking (page 11), which is slightly disturbing. As a matter of fact, the EM does not support the model in my opinion. In all of the EM images presented (control, Kif3a-/-, IFT88-/-) a single basal foot is visible. In the serial sections presented in fig 6b, which are oblique with respect to the basal body longitudinal axis, the tip of the basal foot is probably present in the top section, thus suggesting that the basal foot is normal. Moreover, I think there are even MTs attached to the basal foot of the Kif3a-/- mother centriole. How many serial sections of basal feet have been observed altogether?*

In analysing over 10 serially sectioned cilia per sample in three separate experiments (>30 samples analysed), we did not detect a primary cilia or basal feet in any of the serial sections of mother centrioles in *Kif3a* mutants. We now include three examples of two consecutive serial sections of mother centrioles in WT, *Kif3a*$^{-/-}$, and *Ift88*$^{-/-}$ cells (Figure 7a-c) to demonstrate the presence of 1-2 basal feet in WT and *Ift88* mutants and an absence in *Kif3a* mutant cells. In WT and *Ift88* mutants a small population of microtubules were anchored to the ciliary basal feet and the pericentriolar material surrounding the mother centriole. Due to the loss of basal feet and microtubule anchoring in *Kif3a* mutants the microtubules the reviewer refers to are likely the pool nucleated by the PCM.

*Page 7, bottom : distal rather than subdistal appendages are mentioned.*

We thank the reviewer for pointing out our misuse of the distal rather than subdistal appendages. We have corrected our misreference to subdistal appendages.

*Scale bars should be indicated in all IF figures.*

We appreciate the reviewers suggestion of including scale bars in all IF figures. We have added scale bars to the bottom right corner of all images.

*Reviewer 2:*
*To provide more mechanistic insight on the potential role of Kif3a in the biogenesis of centriole appendages, the authors should perform structure function analysis in wild-type and Kif3a -/- MEFs to look at the ability of various Kif3a truncation mutants to rescue loss of centriole cohesion, cilia formation and basal feet assembly. The ability to restore localization of other components although suggestive of rescue needs to be further substantiated. It would be particular informative if the authors could investigate the role of the motor domain, through point mutations affecting motor activity instead of inferring this from large deletion mutants, in these processes. This would allow them to determine if motor activity is actually required for these processes.*

As suggested by the reviewer, we expressed the Kif3a truncation mutants in *Kif3a*$^{-/-}$ MEFs and examined their effects on centriole cohesion and cilia formation, but aggregation and cellular toxicity prevented us from reaching firm conclusions. Unlike centriole cohesion and cilia formation, which require more than 24 hours to be assayed, p150$^{Glued}$ localization could be restored following six hours of expressing the various Kif3a mutant constructs and prior to aggregation and cell death. We also created two motor dead Kif3a mutants and expressed them in *Kif3a*$^{-/-}$ MEFs. As seen with Kif3a lacking the motor domain, both motor dead Kif3a mutants localized to the centrosome and restored the subdistal appendage localization of p150$^{Glued}$ in *Kif3a*$^{-/-}$ MEFs, arguing that Kif3a organizes the subdistal appendage independent of its motor function. These new data are included in Figure 4f and g.

*In microtubule organization experiment, the authors should consider the role of Ninein in MT anchoring in the interpretation of their results. Having shown that Kif3a and p150 are both required for Ninein localization, the fact that less microtubules were anchored at the*

*centrosomes in Kif3a mutants might be a consequence of Ninein mislocalization rather than a direct role for kif3a in microtubule organization and anchoring.*

To test the possibility that defects in microtubule anchoring are secondary to Ninein mislocalization, we depleted cells of Ninein and assessed microtubule anchoring in a microtubule regrowth assay. Similar to the findings of (Ibi et al, 2011), depletion of Ninein disrupted microtubule anchoring at the centrosome following nocodazole washout. Furthermore, we found that Kif3a localization does not require Ninein, suggesting that the microtubule anchoring defect in *Kif3a* mutants is due to the mislocalization of Ninein, as proposed by the reviewer. These new data are now included in the new Figure 6d and 6f.

*The authors should carefully analyse the relative levels Ninein, p150Glued and Kif3a upon RNAi of each of these proteins and in wild type cells and in the Kif3a -/- MEFs. This could rule-out potential indirect effects of protein stability when one of the other proteins is missing and is necessary to make statements on the ability of different proteins to localize in absence of each other.*

To address the reviewer's concern, we assessed the levels of Ninein, p150$^{Glued}$ and Kif3a in knockdown and knockout cells. We found that there is no change in the levels of these three proteins, suggesting that the localization phenotypes are not due to protein destabilization. These new data are included in Figures 2e, 3g, and 6e.

*The author analyse in detail the distribution of Kif3a on centrioles and show that its distribution is different than other subdistal appendage proteins, including p150 with which it interacts. The authors should explain what exactly this means and how it fits with their interaction data.*

We speculate that the different distributions displayed by Kif3a and p150$^{Glued}$ on the distal centriole suggest that, while a subpopulation of both proteins colocalize in a subdomain of the subdistal appendage region, the majority of centriolar Kif3a and p150$^{Glued}$ do not colocalize or interact, or do so in a dynamic way. This limited colocalization of subdistal appendage proteins has also been observed for Odf2 and Ninein (Ishikawa et al, 2005; Krauss et al, 2008). Similar to the localization and functional relationship between Kif3a and p150$^{Glued}$, Odf2 stains as a single focus at the mother centriole and is required for the localization of Ninein to the subdistal appendage. Unlike subdistal appendage proteins, which are lost upon entry into mitosis (Guarguaglini et al, 2005), Kif3a and Odf2 remain associated with the centrosome throughout the cell cycle (Anderson & Stearns, 2009). Given the constituent localization of Kif3a and Odf2 to mother centrioles, we hypothesize that these two proteins assist in reforming subdistal appendages upon entry into G1. We will pursue live cell imaging of fluorescently tagged versions of these proteins required to test these hypotheses.

*Along these lines, it is curious that the dimmer of the two Centrin dots are the ones that co-label with Kif3a. It is generally accepted that the brighter Centrin structure is the mother centriole so this data appears to contradict what is shown in Figure 1D using mother specific markers. The authors should clarify this.*

As described above in the comments to the first reviewer, we reimaged cells and found that Kif3a associates with the brighter Centrin structure, the mother centriole. These new images are now included in Figure 1c.

*The distribution of Kif3a on mitotic centrioles is intriguing because it is no longer punctate. The authors should comment on whether or not this is consistent with Kif3a being a subdistal appendage marker.*

The loss of punctate Kif3a staining during mitosis correlates with the loss of subdistal appendages (Guarguaglini et al, 2005). One possible technical explanation is that the Kif3a antibody, raised against the p150$^{Glued}$ interacting domain, does not detect the centrosomal population of Kif3a bound by p150$^{Glued}$, resulting in a punctate interphase staining. However during mitosis, p150$^{Glued}$ is no longer at the centrosome therefore the entire

centrosome pool of Kif3a can be detected at the spindle poles, thereby producing an elongated staining. Alternatively, Kif3a, like several other proteins involved in subdistal appendage formation, may shift its localization during mitosis.

*The authors should cite previous work on IFT88 mutants in Chlamydomonas and humans that showed that basal bodies are anchored at the plasma membrane in absence if IFT88 as it is related to their observation that IFT88 mutants still have basal feet.*

Thank you for the recommendation. We have now included references to the work by Greg Pazour demonstrating that basal bodies in *Ift88* mutant mice and Chlamydomonas dock to the plasma membrane (Pazour et al, 2000).

*Thank you again for your extensive help with this manuscript. Your efforts and suggestions have substantially improved our work, and I deeply appreciate your insights. Please let me know if I can provide any additional information or answer any further questions.*

Yours sincerely,

Editorial Decision 04 December 2012

Your paper has been re-reviewed by one of the original referees with no further comments and I am pleased to inform you that it has been accepted for publication in the EMBO Journal, pending that you include some minor changes as described below:

- Error bars in graphs are not defined. Their definition (standard deviation, confidence interval, standard error, etc.) must be added in the figure legend.

- Please remove the supplementary text from the article file and summarize all supplementary information within one single PDF in its final format (it will not be copy-edited before publishing). This includes not only supplementary figures, but also supplementary text (figure legends and supplementary materials and methods). We would also need the final main article text as a Word file. Please send me all the modified files by e-mail and we will upload them into the system for you.

I would also like to mention that, as a novel initiative in The EMBO Journal, we now encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. Although optional at the moment, would you be willing to provide a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figures? The PDF files should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The files will be published online with the article as supplementary "Source Data" files. If you have any questions regarding this initiative do not hesitate to contact me.

If you have any questions, please do not hesitate to contact me. Thank you for your contribution to The EMBO Journal and congratulations on a successful publication.

Yours sincerely,
Editor,
The EMBO Journal