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# Msd1/SSX2IP-dependent microtubule anchorage ensures spindle orientation and primary cilia formation

Akiko Hori, Chiho Ikebe, Masazumi Tada and Takashi Toda

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**Review timeline:**

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|---|---|
| Submission date: | 29 August 2013 |
| Editorial Decision: | 23 September 2013 |
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| Editorial Decision: | 04 November 2013 |
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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.)

Editor: Esther Schnapp

1st Editorial Decision 23 September 2013

Thank you for the submission of your manuscript to our journal. We have now received the full set of referee reports that is pasted below.

As you will see, all referees agree that the findings are of interest. However, they also suggest additional experiments to strengthen the findings and to compare them to a recent publication reporting similar results. All referees point out that the work by Barenz et al should be discussed in more detail and that the differences should be worked out more clearly. Referee 2 further adds that it should be examined whether the siRNAs used by Barenz et al result in the same phenotypes. All referees also pinpoint a number of missing, important quantifications, statistical analyses and experimental details that must all be provided. Finally, after cross-commenting on each others' reports, referee 2 feels that the role of dynein and microtubules in the transport of SSX2IP to centrosomes and the effect of SSX2IP on spindle positioning does not need to be further addressed, and I agree.

 

Given these comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as mentioned above and in their reports) must be fully addressed and their suggestions taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Also, the revised manuscript may not exceed 30,000 characters (including spaces and references) and 5 main plus 5 supplementary figures, which should directly relate to their corresponding main figure. The current character count largely exceeds our limits, and the manuscript text therefore needs to be substantially shortened. EMBO reports papers use a numbered reference style, and changing reference style will help in shortening the text. You can further combine the results and discussion section, which may eliminate some redundancy that is inevitable when discussing the same experiments twice. Parts of the materials and methods can also be moved to the supplementary information, but please note that the materials and methods essential for the understanding of the experiments described in the main manuscript file must remain in the main methods section. Regarding the number of figures, the referees suggest that figures 2 and 3, figures 5 and 6, and figures 7 and 8 could be combined, and that these (or part of these) together with figure 4 should be kept in the main manuscript file. Confirmatory data can be moved to the supplementary information. However, please feel free to re-organize the manuscript text and figures as you deem appropriate. While we cannot allow more than 5 main figures, the number of supplementary figures is more flexible.

Regarding data quantification, can you please specify the number "n" for how many experiments were performed (or how many cells used), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends? This information must be provided in the figure legends.

When submitting your revised manuscript, please include:

A Microsoft Word file of the manuscript text, editable high resolution TIFF or EPS-formatted figure files, a separate PDF file of any Supplementary information (in its final format), a letter detailing your responses to all the referee comments, and a two sentence summary of your findings and their significance.

We recently decided to offer the authors the possibility to submit "source data" with their revised manuscript that will be published in a separate source data file online along with the accepted manuscript. If you would like to use this opportunity, please submit the source data (for example entire gels or blots, data points of graphs, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one file per figure.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have any further questions or comments regarding the revision.

 

REFEREE REPORTS:

Referee #1:

The work of Toda and colleagues describes the functional analysis of Msd1 proteins at the centrosome. Previous studies from the same lab had analysed the F. Yeast homologue, which had a mitotic localization. Here the authors show that hMSD1 localises throughout the cell cycle and, this localization is similar to previously described centriolar satellites. To further prove this point, the authors use nocdazole depolymerising experiments and immunoprecipitation experiments and confirm that Msd1 is a component of centriolar satellites. The authors also show that Msd1 plays a role in MT anchoring, but not nucleation and that this role does not dependent on Ninein. Beautiful rescue experiments that targeted the Msd1-gtubulin domain to the centrosome (PACT) rescue the phenotype. The authors then found a role for Msd1 in astral MT anchoring and spindle positioning. Moreover, they also found a role for hMsd1 in cilia formation in vivo, in zebrafish.
Overall this is a very nice study that shows a novel function for a yet uncharacterised protein and so deserves publication by EMBO reports. The only suggestion I have is that the authors should complete their method section and inform the reader about the number of cells analysed for the knock down experiments, rescue experiments and the statistic analysis performed in each case.

Referee #2:

Review of manuscript by Hori et al. "Msd1/SSX2IP-dependent microtubule anchorage ensures spindle orientation and primary cilia formation"

1. Does this manuscript report a single key finding? YES

SSX2IP is a MT anchoring factor at centrosomes that controls astral MT organization.

2. Is the reported work of significance (YES), or does it describe a confirmatory finding or one that has already been documented using other methods or in other organisms etc (NO)? YES and NO

Some of the results confirm data from a recent study, but most of the results are new.

3. Is it of general interest to the molecular biology community? YES

The study analyzes the role of an important centrosome regulator in MT organization and spindle function.

4. Is the single major finding robustly documented using independent lines of experimental evidence (YES), or is it really just a preliminary report requiring significant further data to become convincing, and thus more suited to a longerformat article (NO)? YES

The study by Hori et al. analyzes the role of Msd1/SSX2IP in the organization of centrosomal microtubules in both cultured cells and in zebrafish. This is an important paper that expands previous work on SSX2IP, which has been described as an oncogene that drives tumor progression and metastasis, possibly through a function in centrosome maturation/structure. The current work suggests that SSX2IP functions by anchoring MTs at centrosomes rather than centrosome maturation and that this is not only important for mitotic spindle assembly but also for the formation of cilia. The manuscript is well written and the data is of high quality. It would require little additional work if it would not have to be compared to a recent, very similar study on the function of SSX2IP (Barenz et al., JCB, 2013). Some of the data in the current study is simply confirmatory; other data is new or contradicts the previous work. The paper would benefit from highlighting these differences in the text and addressing some of the issues experimentally. If contradictory results are obtained the authors should take a stand against the previous study rather than discussing differences in the siRNAs as a possible explanation and leaving the interpretation to the reader.

 

Major issues:

1) Considering that the work by Barenz et al. was published in July 2013 it should be mentioned in the introduction and/or results (e.g. p. 5), rather than only briefly at the very end, in the discussion. I also suggest a more detailed discussion of the Barenz paper, to highlight differences between the two studies.

2) p. 7: "...simultaneously localises to the core centrosome via physical interaction with the γ-TuC." This sentence is misleading since there is no evidence that gTuC recruits SSX2IP to the centrosome.

3) Fig. S2: One important difference compared to the study by Barenz et al. is that the authors of the current manuscript do not see any effect of SSX2IP depletion on centrosomal levels of gtub and other PCM proteins, and no impairment of nucleation activity. The authors should test the siRNAs used by Barenz et al. to rule out siRNA-specific effects. They should compare the levels of depletion between the different siRNAs by westernblot and test whether the same results are obtained also with the siRNA used by Barenz et al.

4) Fig. 4E: The presentation of the data (text and figure) is misleading since rescue with full length SSX2IP is not shown in the same experiment for comparison (Fig. 4C shows that rescue with full length protein is much better than with the SSX2IP-C-PACT construct). Therefore the centrosome targeting via PACT only partially recues suggesting that the interaction with PCM1 might also be important for full functionality.

5) Spindle pole fragmentation and cilia defects can also be explained by abnormal centriole numbers. The authors should quantify centriole number in control and SSX2IP-depleted cells.

6) Fig. S4: The aster forming activity at mitotic centrosomes in the regrowth assay should be quantified to confirm that lack of SSX2IP does not affect nucleation.

Minor issue:
7) "SSX2IP" is the official gene name and the name used in the literature. The authors might want to consider using this name instead of "hMsd1".

Referee #3:

In this manuscript the authors report that the human orthologue of yeast Msd1/SSX2IP protein is required for microtubule anchorage to the centrosome. Msd1 is shown to be crucial for several aspects of cell-cycle by orchestrating proper microtubule organization both during interphase as well as in mitosis. By analyzing intra-cellular localization and interaction of several N and C-terminal deletion constructs of Msd1, the authors identify γ-tubulin and PCM1 interacting region of Msd1. In addition, artificially targeting of γ-tubulin interacting region of Msd1 to the centrosome, the authors convincingly show that this fusion protein is sufficient to rescue microtubule dependent processes caused upon endogenous Msd1 depletion. Moreover, Msd1 was also shown to be critical for proper spindle positioning in HeLa cells, cilia formation in epithelial cells and its knockdown also causes developmental defects in Zebrafish.
Overall, this is an interesting manuscript and the data shown in this manuscript is of good quality. Therefore, this manuscript should be of interest to many cell and developmental biologists, particularly working in the field of mitosis, centrosome and on cell cycle. While these results are interesting, I feel that it should be strengthened by additional experiments to consolidate the conclusions drawn by the authors.
Major Concerns:
1. The authors claim that Msd1 is delivered to the centrosome in a microtubules and dynein dependent manner. The authors put a lot of emphasis on this finding throughout the manuscript and mention this in abstract, discussion as well as in their working model. However, the data presented in the current manuscript regarding the role of microtubules and dynein (Supplementary figure 1D and IE) are not very well controlled and in general too weak to draw such strong conclusions. I therefore feel that additional experiments (see below) should be performed.

 

- Short term microtubule de-polymerization either using Nocodazole or expression of p50-dynamitin to block dynein function indicates that Msd1 do not localizes to the centriolar satellite under such condition, however it still localizes to centrosomes. Thus, I suggest that long term microtubules de-polymerization experiment must be conduct together with other means of inhibiting dynein function (e.g. using Ciliobrevin) to strengthen the importance of dynein and microtubules in the localization of Msd1 to the centrosome. In addition quantification of this phenotype must be provided, which is currently missing in the manuscript.

2. The authors show by various mean an impact on astral microtubules in metaphase cells upon Msd1 depletion; however, it is unclear to me why there is no influence of Msd1 depletion on spindle microtubules. On the contrary, it seems that there are more spindle microtubules in Msd1 depleted cells (Figure 6A). I suggest that intensity of spindle microtubules must be quantified and thus must be reported in the revised manuscript. In addition, astral microtubules quantification must be performed in a condition where Msd1-C-PACT is expressed in Msd1 depleted cells.

3. Due to apparent influence on astral microtubules upon Msd1 depletion, the role of Msd1 on spindle positioning on uniform substrate is not surprising. Since either loss of cortical pulling forces or excess pulling forces perturb spindle positioning on uniform substrate, I would advice to monitor spindle positioning by conducting live-imaging experiment using cells expressing fluorescently labeled microtubules/chromosome to better understand the nature of spindle positioning phenotype in such condition.

Minor points:

1. Using two different siRNAs in RPE cells, Bärenz et al., 2013 has recently shown that Msd1 depletion affects γ-tubulin levels as well as causes its fragmentation during mitosis. In the current manuscript, authors did not observe any change in γ-tubulin levels and/or its fragmentation. Though authors have acknowledge this finding of Bärenz et al., 2013 in their discussion and mentioned this apparent discrepancy could be because of different siRNAs sequences used in these two study. I failed to understand their reasoning for such discrepancy, thus I suggest this must be clarified experimentally. It could well be that this phenotype is cell-type specific or more extensive depletion of Msd1 give such phenotype.
2. It would be interesting to see if the effect of Msd1 depletion on cilia could also be rescued by expression of Msd1-C-PACT.
3. At few instances molecular weight on the western blots, scale bars values in the figure legends are missing, it must be corrected.
4. It was not clear to me on which substrate spindle positioning assays were performed, thus this must be clarified as well

1st Revision - authors' response 28 October 2013

**to the editor:**

Editing and shorting of the manuscript

The total number of figures is now five with six Supplementary figures; Supplementary Figs S1-5 are related to corresponding Figs 1-5 and Fig S6 deals with the issue with regards to recently published related work by Barenz et al. (2013) (see below for details). The number of characters is 22,674 (the main text plus references), 7,831 (figure legends) and 3,016 (Methods).

Data quantification

As requested, we have provided detailed information on statistical analysis of the data in each figure legend, including how many experiments are performed (n=), the number of samples analysed, error bards (SD) and the test used to calculate p-value (Student's t test, two-tailed). In addition, overall summary of statistical analysis is given in the Methods section as "Statistical data analysis".

 

**Referee #1:**

Thank you very much for his/her very positive evaluation of our work.

The only suggestion I have is that the authors should complete their method section and inform the reader about the number of cells analysed for the knock down experiments, rescue experiments and the statistic analysis performed in each case.

As described above in the reply to the editor, we have now provided information of *"the number of cells analysed for the knock down experiments, rescue experiments and the statistic analysis performed in each case"* in the Method section as well as in each figure legend.

**Referee #2:**

We appreciate this referee's thorough, constructive comments.

Major issues:

1) Considering that the work by Barenz et al. was published in July 2013 it should be mentioned in the introduction and/or results (e.g. p. 5), rather than only briefly at the very end, in the discussion. I also suggest a more detailed discussion of the Barenz paper, to highlight differences between the two studies.

We mentioned, as suggested, the paper by Barenz et al. (2013) in page 4 (the very first part of the Results and Discussion section) and later in pages 9 and 10 discussed the primary roles of hMsd1/SSX2IP in relation to the two studies performed by Barenz et al. and us. Furthermore, more detailed descriptions and explanations including the similarities and differences based upon our new experimentations have been given in Supplementary Note 2 and Fig S6 (see below for more details). We think that the differences between the two groups (centrosome fragmentation vs microtubule anchoring defects) stem from the different timings of phenotype observations; we observed phenotypes **48 h** after siRNA treatment, whilst Barenz did so after **78-88 h** (Barenz et al. 2013). As shown in Supplementary Fig S6B-D (new data), whilst microtubule defects were already obvious after 48 h and not augmented even after 96 h, the centrosome fragmentation phenotype became apparent only after 96 h later (we also describe this point in response to the referee #3 minor point 1).

We think that the centrosome fragmentation phenotype could be a secondary consequence derived from the primary microtubule defects, as several γ-tubulin attachment factors are reportedly transported to the centrosome via centriolar satellites and microtubules. We consider that microtubule disorganisation caused by hMsd1/SSX2IP depletion (**at 48h**) would then lead to failure of γ-tubulin attachment to the centrosome, resulting in centrosome fragmentation (**at 78 h** and later).

2) p. 7: "...simultaneously localises to the core centrosome via physical interaction with the γ-TuC." This sentence is misleading since there is no evidence that gTuC recruits SSX2IP to the centrosome.

We agree with this point. As suggested, we have removed *"via physical interaction with the γ-TuC"*.

3) Fig. S2: One important difference compared to the study by Barenz et al. is that the authors of the current manuscript do not see any effect of SSX2IP depletion on centrosomal levels of gtub and other PCM proteins, and no impairment of nucleation activity. The authors should test the siRNAs used by Barenz et al. to rule out siRNA-specific effects. They should compare the levels of depletion between the different siRNAs by westernblot and test whether the same results are obtained also with the siRNA used by Barenz et al.

In response to this referee's request, we performed immunoblotting to see the efficacy of the three different hMsd1/SSX2IP siRNAs used by us (hMsd1 siRNA) and Barenz et al. (No.1 and No.2). As shown in Supplementary Fig S6A, these three siRNAs knocked down hMsd1/SSX2IP protein levels with almost the same effectiveness; only a very modest amount of residual hMsd1/SSX2IP protein was observed in RPE-1 cells 48 h after No.2 siRNA treatment.

4) Fig. 4E: The presentation of the data (text and figure) is misleading since rescue with full length SSX2IP is not shown in the same experiment for comparison (Fig. 4C shows that rescue with full length protein is much better than with the SSX2IP-C-PACT construct). Therefore the centrosome

 

targeting via PACT only partially recues suggesting that the interaction with PCM1 might also be important for full functionality.

We have now added the suppression data of interphase microtubule disorganisation by hMsd1/SSX2IP-C-PACT as well as that by full-length hMsd1/SSX2IP, and compared their rescue efficiencies. As shown in new Fig 3C, despite that hMsd1/SSX2IP-C-PACT looked to rescue less efficiently, statistical tests showed no significant differences (p=0.06). That is also true for suppression of spindle defects by these two constructs (Fig 4E, G and H; p-values are 0.57, 0.1 and 0.5 respectively).

5) Spindle pole fragmentation and cilia defects can also be explained by abnormal centriole numbers. The authors should quantify centriole number in control and SSX2IP-depleted cells.

As requested, we observed the number of centrin-GFP (a centriole marker) in hMsd1/SSX2IP siRNA-treated RPE-1 cells. We did not see any abnormalities of centriole numbers (the number is less than 4). This data is shown in Supplementary Fig S5A and B.

6) Fig. S4: The aster forming activity at mitotic centrosomes in the regrowth assay should be quantified to confirm that lack of SSX2IP does not affect nucleation.

We quantified intensities of aster microtubules emanating from the centrosome in the regrowth assay during mitosis (5 min time-point). As shown in Supplementary Fig S4H, nucleation activities are not compromised in hMsd1/SSX2IP-depleted cells.

Minor issue:

7) "SSX2IP" is the official gene name and the name used in the literature. The authors might want to consider using this name instead of "hMsd1".

We agree that the name of SSX2IP was first given. In this manuscript, in many places (subheadings in each section and captions of figure legends etc) we used hMsd1/SSX2IP. I think that the readers will not be confused with nomenclatures. Whether or not SSX2IP will be used hereafter in the field is another issue. We would like to ask to use hMsd1/SSX2IP in this paper in order to emphasise the structural and functional conservations of the Msd1-SSX2IP family members from fission yeast to zebrafish and humans.

**Referee #3:**

We also thank this referee for his/her critical, thoughtful comments.

1. The authors claim that Msd1 is delivered to the centrosome in a microtubules and dynein dependent manner. ,,,,,,,,

- Short term microtubule de-polymerization either using Nocodazole or expression of p50-dynamitin to block dynein function indicates that Msd1 do not localizes to the centriolar satellite under such condition, however it still localizes to centrosomes. Thus, I suggest that long term microtubules de-polymerization experiment must be conduct together with other means of inhibiting dynein function (e.g. using Ciliobrevin) to strengthen the importance of dynein and microtubules in the localization of Msd1 to the centrosome. In addition quantification of this phenotype must be provided, which is currently missing in the manuscript.

We think that this is an important point but is beyond the scope of the current study. We will address this issue in the future. The editor and referee #2 also feel this way.

2. The authors show by various mean an impact on astral microtubules in metaphase cells upon Msd1 depletion; however, it is unclear to me why there is no influence of Msd1 depletion on spindle microtubules. On the contrary, it seems that there are more spindle microtubules in Msd1 depleted cells (Figure 6A). I suggest that intensity of spindle microtubules must be quantified and thus must be reported in the revised manuscript. In addition, astral microtubules quantification must be performed in a condition where Msd1-C-PACT is expressed in Msd1 depleted cells.

Thank you very much for pointing out this important issue. In response to this referee's suggestion, we analysed spindle microtubule intensities, and as this referee suspected, found that in fact they were reduced by ~40%. This data is now shown in Fig 4H. In addition, we quantified

 

intensities of astral microtubules in hMsd1-C-PACT-transfected, hMsd1-depleted cells. Quantification showed that the reduced intensities of astral microtubules are rescued by hMsd1-C-PACT (shown in Fig 4G).

3. Due to apparent influence on astral microtubules upon Msd1 depletion, the role of Msd1 on spindle positioning on uniform substrate is not surprising. Since either loss of cortical pulling forces or excess pulling forces perturb spindle positioning on uniform substrate, I would advice to monitor spindle positioning by conducting live-imaging experiment using cells expressing fluorescently labeled microtubules/chromosome to better understand the nature of spindle positioning phenotype in such condition.

This is an important issue to be addressed in the future, but we feel that it is beyond the scope of this study and the editor and referee #2 also think so.

**Minor points:**

1. Using two different siRNAs in RPE cells, Bärenz et al., 2013 has recently shown that Msd1 depletion affects γ-tubulin levels as well as causes its fragmentation during mitosis. In the current manuscript, authors did not observe any change in γ-tubulin levels and/or its fragmentation. Though authors have acknowledge this finding of Bärenz et al., 2013 in their discussion and mentioned this apparent discrepancy could be because of different siRNAs sequences used in these two study. I failed to understand their reasoning for such discrepancy, thus I suggest this must be clarified experimentally. It could well be that this phenotype is cell-type specific or more extensive depletion of Msd1 give such phenotype.

This is the same issue as (3) raised by referee 2. We reiterate our (new) results and responses. We believe that the phenotypic differences between the two groups are attributed to the timing of phenotype observations, not siRNAs used by Bärenz et al. and us. In this study, we observed microtubule phenotypes of hMsd1/SSX2IP-depleted cells **48 h after** siRNA treatment. In contrast, Bärenz et al. started their observations much later (**78 h**). Under their condition, centrosomal defects including centrosome fragmentation appeared at **88 h** time point (Bärenz et al., 2013); by the way, they did not observe interphase microtubule morphologies.

In response to referees' request, we observed spindle microtubules and centrosomes 48 h and 96 h after siRNA treatment. As shown in Supplementary Fig S6B-D, the appearance of centrosome fragmentation was time-dependent; ~20% at 48 h and ~40% at 96 h. In contrast, interphase microtubule disorganisation and spindle tilt were already evident at 48 h (70-80%) and the percentage was not increased at 96 h time point. We think that the centrosome fragmentation phenotype could be a secondary consequence derived from primary microtubule defects, as several γ-tubulin attachment factors such (eg. Pericentrin) are reported to be transported to the centrosome via centriolar satellites and microtubules. Alternatively, albeit not mutually exclusive, hMsd1/SSX2IP might be involved directly in centrosome maturation, as 20% of mitotic hMsd1/SSX2IP-depleted cells displayed the centrosome fragmentation phenotype at earlier time point. This notion is described in the main text (pages 9 and 10) and discussed in more detail in Supplementary Information (Supplementary Note 2).

2. It would be interesting to see if the effect of Msd1 depletion on cilia could also be rescued by expression of Msd1-C-PACT.

As suggested, we performed this experiment. hMsd1-C-PACT did rescue cilia defects, which is shown in Supplementary Fig 6C-F.

3. At few instances molecular weight on the western blots, scale bars values in the figure legends are missing, it must be corrected.

Thank you very much for pointing out our mistakes. Molecular weight and scale bars values have been added in all appropriate places.

4. It was not clear to me on which substrate spindle positioning assays were performed, thus this must be clarified as well.

We did not treat coverslips with fibronectin. The media we used [DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS)] contained fibronectin, and this seems sufficient for cells to adhere to the substratum. As a control and to verify this notion, we performed spindle

 

orientation assay in cells plated on fibronectin-coated coverslips, and obtained the identical results. This is described in page 7 and the data is shown in Supplementary Fig S4D.

We hope that revisions suffice referees' comments and requests, and the revised manuscript is now suitable for publication in EMBO Report.

2nd Editorial Decision 04 November 2013

Thank you for the submission of your revised manuscript. We have now received the enclosed report from referee 2, who was asked to assess it. As you will see, while referee 2 is overall happy with the revised study, s/he remains of the opinion that it is very important to examine the centrosomal nucleation activity by quantifying the intensities of MT asters in the presence and absence of Msd1. I would therefore like to give you the exceptional opportunity to provide this missing information, so that we can proceed with the official acceptance of your manuscript.

I also need to point out that the character count exceeds our limit, and that the manuscript text needs to be further shortened. We also do not allow supplementary notes in the SI, which need to be taken out. In order to shorten the main text, commonly used materials and methods can be moved to the SI, and if all of the statistical information is provided in the figure legends, this part can also be removed from the methods.

I also would like to suggest to delete the last sentence of the abstract, as you do not investigate the role of Msd1 in ciliopathies or cancer, and instead modify it to:

We propose that the Msd1 family comprises conserved microtubule-anchoring proteins that are required for ciliogenesis.

Please let me know whether you agree with this change.

I look forward to seeing a new revised version of your manuscript as soon as possible.

REFEREE REPORT:

Referee #2:

The authors have addressed most of my concerns in their revised manuscript.
There is one issue though that needs further examination:

In point 6) of my review I asked for quantification of the centrosomal nucleation activity after SSX2IP RNAi.

"6) Fig. S4: The aster forming activity at mitotic centrosomes in the regrowth assay should be quantified to confirm that lack of SSX2IP does not affect nucleation."

In their rebuttal the authors claim to have perfomed this experiment:

"We quantified intensities of aster microtubules emanating from the centrosome in the regrowth assay during mitosis (5 min time-point). As shown in Supplementary Fig S4H, nucleation activities are not compromised in hMsd1/SSX2IP-depleted cells."

 

However, in Fig. S4H the authors have quantified the percentage of cells with asters, but not the intensities of MT asters in these cells. The presence of an aster alone is not a quantitative assessment of nucleation activity, since the asters could have differences in MT density, for example. The nucleation activity can only be determined by measuring intensities of MT staining. This can be done in a circular area with a fixed diameter around centrosomes.

After re-reading the manuscript I noticed that such quantification has also not been performed in interphase centrosomes (Fig. 2). However, this is not as crucial, since the authors later focus on the mitotic centrosome defects. Here, the nucleation activity needs to be tested, since reduced nucleation could also account for the lack of astral MTs in SSX2IP-depleted cells.

2nd Revision - authors' response 05 November 2013

**To the editor:**
The number of total characters (with space and references) is 29,868.

**Referee #2:**
However, in Fig. S4H the authors have quantified the percentage of cells with asters, but not the intensities of MT asters in these cells. The presence of an aster alone is not a quantitative assessment of nucleation activity, since the asters could have differences in MT density, for example. The nucleation activity can only be determined by measuring intensities of MT staining. This can be done in a circular area with a fixed diameter around centrosomes.

In response to referee #2's point, we quantified microtubule intensities of mitotic asters around the centrosome upon microtubule regrowth assay. As shown in new Supplementary Fig. S4H (left), microtubule intensities are similar between control and Msd1 siRNA treated cells ($p$=0.24).

3rd Editorial Decision 08 November 2013

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

 