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HARTONO UNM <hartono@unm.ac.id>

Notification to co-authors of submission to Cell Division CDIV-D-19-00011

1 message

Cell Division - Editorial Office <em@editorialmanager.com> 1 April 2019 at 15:57
Reply-To: Cell Division - Editorial Office <parthiban.gurusamy@springer.com>
To: Hartono Hartono <hartono@unm.ac.id>

CDIV-D-19-00011
Nucleoporin Nup58 localized at the centrosomes and mid-bodies during mitosis
Richard Wong; Hartono Hartono; Masaharu Hazawa; Kee Siang Lim; Firli R.P. Dewi; Akiko Kobayashi

Dear author:

You are receiving this email because you have been listed as an author on a manuscript recently submitted to Cell Division. The manuscript details are below.

Title: Nucleoporin Nup58 localized at the centrosomes and mid-bodies during mitosis
Authors: Richard Wong; Hartono Hartono; Masaharu Hazawa; Kee Siang Lim; Firli R.P. Dewi; Akiko Kobayashi
Corresponding author: Prof. Richard Wong

If you are not aware of the submission, or if you should not be listed as contributing author, please notify the Editorial Office. Contact details for the Editorial Office are available under "Contact Us" on the journal website.

Kind regards,

Editorial Office
Cell Division
https://celldiv.biomedcentral.com/

UNM

HARTONO UNM <hartono@unm.ac.id>

Fwd: Your submission to Cell Division - CDIV-D-19-00011

1 message

Richard Wong <rwongkanazawa@gmail.com> 23 April 2019 at 15:59
To: "HARTONO, S.Si, S.Pd, M.Biotech UNM" <hartono@unm.ac.id>, 羽澤勝治 <masaharu.akj@gmail.com>, Lim keesiang <xiscolim@gmail.com>, firly rahmah <firlyrahmah@gmail.com>, 小林亜紀子 <akoba@staff.kanazawa-u.ac.jp>

Dear Hartono,

Again, major revision.
I suggested to stop Plk1 story at this moment.

Richard

Dear Firli,
To speed up the revision process,
can you contact to Hartono and try to explain to him what are required experiments ?

Many thanks in advance.

Best,
Richard

---------- Forwarded message ---------
From: <rwong@staff.kanazawa-u.ac.jp>
Date: 2019年4月23日(火) 16:41
Subject: Fwd: Your submission to Cell Division - CDIV-D-19-00011
To: <rwongkanazawa@gmail.com>

-------- 元のメッセージ --------
件名: Your submission to Cell Division - CDIV-D-19-00011
日付: 2019-04-23 16:40
発信者: "Cell Division - Editorial Office" <em@editorialmanager.com>
宛先: "Richard Wong" <rwong@staff.kanazawa-u.ac.jp>
返信先: "Cell Division - Editorial Office"
<parthiban.gurusamy@springer.com>

CDIV-D-19-00011
Nucleoporin Nup58 localized at the centrosomes and mid-bodies during mitosis
Hartono Hartono; Masaharu Hazawa; Kee Siang Lim; Firli R.P. Dewi; Akiko Kobayashi; Richard Wong
Cell Division

Dear Richard,

Your manuscript "Nucleoporin Nup58 localized at the centrosomes and mid-bodies during mitosis" (CDIV-D-19-00011) has been assessed by our reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication

in Cell Division, once you have carried out some essential revisions suggested by our reviewers.

Their reports, together with any other comments, are below. Please also take a moment to check our website at https://www.editorialmanager.com/cdiv/ for any additional comments that were saved as attachments.

Once you have made the necessary corrections, please submit a revised manuscript online.

Your username is: wong2017

If you forgot your password, you can click the 'Send Login Details' link on the EM Login page at https://www.editorialmanager.com/cdiv/.

Please include a point-by-point response within the 'Response to Reviewers' box in the submission system and highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript. Please ensure you describe additional experiments that were carried out and include a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that your revised manuscript conforms to the journal style, which can be found in the Submission Guidelines on the journal homepage.

The due date for submitting the revised version of your article is 22 Jun 2019.

Please note, if your manuscript is accepted you will not be able to make any changes to the authors, or order of authors, of your manuscript once the editor has accepted your manuscript for publication. If you wish to make any changes to authorship before you resubmit your revisions, please reply to this email and ask for a 'Request for change in authorship' form which should be completed by all authors (including those to be removed) and returned to this email address. Please ensure that any changes in authorship fulfil the criteria for authorship as outlined in BioMed Central's editorial policies (http://www.biomedcentral.com/about/editorialpolicies#authorship).

Once you have completed and returned the form, your request will be considered and you will be advised whether the requested changes will be allowed.
By resubmitting your manuscript you confirm that all author details on the revised version are correct, that all authors have agreed to authorship and order of authorship for this manuscript and that all authors have the appropriate permissions and rights to the reported data.

Please be aware that we may investigate, or ask your institute to investigate, any unauthorised attempts to change authorship or discrepancies in authorship between the submitted and revised versions of your manuscript.

We look forward to receiving your revised manuscript soon.

Thank you.

Best wishes,

Philipp Kaldis, PhD
Cell Division
https://celldiv.biomedcentral.com/

Reviewer reports:

Reviewer #1: In this report, Hartono et al. further confirm the implication of some nucleoporins in different mitotic mechanisms. Using confocal and time lapse microscopy, authors show the localization of Nup58 during the cell cycle and highlight its spindle midbody relocalization during the citokinesis. They further assessed the importance of Nup58 on the centrosome and the midbody by knocking it down. Although the displayed experiments are well designed, and correctly explained in the text and legends, the Nup58 knockdown lacks of in depth analysis that will help to understand correctly the importance of this nucleoporin during mitosis progression. The previous report by the authors has revealed the importance of Nup62 on the centrosome homeostasis; therefore the action of Nup58, binding partner of Nup62, is not unexpected at the difference of its action on the midbody. To separate both, authors should analyse deeper this difference and understand what is the part of the
phenotype due to the Nup62 complex or from each individual protein.
In figure 4A, the authors are not showing Nup62. Is Nup62 like Nup58 relocalizing to the midbody?
In figure 5d, the knock down (KD) of Nup58 induces centrosome abnormalities but the description of this abnormalities is missing. Authors should break down the different abnormalities and described them further. Further comparison with Nup62 KD should also be discussed .
Using their timelapse images, authors could analyse deeper the phenotype of Nup58 KD. Are cells presenting centrosome abnormalities also presenting midbody issues? How long cells with such abnormalities remain in mitosis? With what outcome?
In this same idea, how does Nup58 KD affect Nup62 on the centrosome. Using both immunofluorescence and immunoprecipitation, authors could evaluate whether the decrease of Nup58 is affecting entirely the complex or is independent of Nup62.

Line 60: verify the punctuation and parentheses " mitosis (10-13) (for review see: (14-17) ".
Line 62 : "Nup358 mitotic functions", the sentence sounds wrong and needs to be verified.
Line 70: re-phrase this sentence
Figure 2: replace the arrow correctly in the last merged panel with SAS IF .

Reviewer #2: The manuscript by Hartono et al is a qualitative examination of the localisation of the Nup58 protein during primarily mitosis and cytokinesis. The manuscript is lacking quantitative data and suffers from significant bias, misinterpretation and over-statement of results. These should be corrected, along with additional quantitation nearly all data. Where quantitation is performed it is currently insufficient. Similarly, confocal microscopy images need to show all z-planes especially when claims of protein absence are being made.

Specific issues and concerns detailed below:

Some curious turns of phrase, long sentences and missing words throughout the manuscript. Additional proof reading needed before publication.
Some examples:

Line 22: Grammar, missing 'are' ???

Line 60: Need to define what CM is.

Line 62: missing 'have'

Line 80: 'down modulation'??? assume you mean knockdown

Figure 1: Why were cells synchronised? There is no quantification of mitotic cells or similar so it makes no sense to introduce potential artefacts when you could quite easily capture all phases of the cell cycle in a simple asynchronous population.
Also, its clear that only a very small section in z is shown, likely only a single z-plane. Why is this? Wouldn't a maximum projection here be more appropriate, especially for mitotic cells?

Also PHEM buffer or similar should really be used here instead of PBS when fixing cells with PFA and staining for microtubules.
Finally, suggest using a colour palette that is not Red-Green i.e one that is colour blind appropriate.

Line 130: Nup58 'is' localised

Figure 2: what are the numbers for in panel a and b? There is no quantification here so these are meaningless. Also again a single z-plane in likely shown. Given that small objects like centrosomes are often at different planes especially in 'thick' mitotic cells, it might be helpful here to show a max projection to ensure that no information is being obscured. Especially when you are trying to say that there is an absence of staining for a protein at a particular stage of mitosis.

Figure 2C: Why is ninein coming down with IgG?

Figure 3A: Why is there no signal in the GFP lane for Nup58-gfp, surely a generic GFP antibody should recognise the tagged version?

Figure 3B: this is mis-leading and a witness-leading figure. It should be removed, as it gives the impression that the GFP tagged protein is localised to all of these locations.

Figure 3D Line 152: There is no evidence shown in these images that Nup58-gfp is localised to centrosomes at any point. The authors should be honest here and state that the localisation is near identical to the control gfp vector. With the exception of late-midbody accumulation.
Line 158: remove 'strongly', this is an overstatement, data here is qualitative at best and does not match the IF data shown previously. Notably, the nuclear membrane and centrosome localisation data is not conserved.

Figure 4: Why is this not before Figure 3? Seems strange to switch from IF to gfp-tag and then back to IF? More logical to keep like data together.

Figure 4A: Is interphase the correct control here? I would have thought that other parts of the fractionation process would have been more informative here. Additional positive and negative control proteins should also be blotted for to confirm specificity of fractionation.

Figure 4c-f: avoid red-green LUTs and again stating of n=21 in one image is irrelevant as there is no quantitation done at all here. Only single image qualitative data is presented.

Figure 5a: again it's not clear why synchronisation is needed here, there is not explanation and the mitotic defects could have been scored of asynchronous cells just as easily, without the confounding issues that synchronisation has on centrosome and cell cycle.

Fig 5b: there is no quantification of blots done here so statements about significance and 85% knockdown should be removed, or better yet, quantification of western data from 3 independent experiments shown. The knockdown of Nup62 and Sas-6 need to be better explained here and not dismissed so quickly.

Fig 5c: again, only single x-plane shown hence the possibility is that details are being obscured. A full stack-max projection is needed here. Similarly, there are no explanation of the types of defects observed, these need to be detailed and ideally separated out into individual phenotypes here.

Fig 5g-h: time to abscission has a very large variation in cells under normal conditions, this phenomena and huge variation needs to be accounted for in 5g. This figure needs to
a) show all data points as a scatter/box-plot and
b) to at the very least count at least 50 cells per condition.
I am not sure how significance was reached in this figure given that the error bars are so large and over-lap and the number of cells counted so low (n=15/16).

The discussion needs to examine the differences between the gfp-tagged and IF data, especially with regards to the fact that the centrosome and nuclear pore localisations do not appear to be retained in the gfp-tagged version. Some more honest appraisal and less biased analysis of the data is also needed.

Reviewer #3: In this manuscript, Hartono et al. study the role of nucleoporin Nup58 in cell division. The authors start by investigating the localization of Nup58 in different mitotic stages, and observe enrichment at centrosomes during metaphase/anaphase as well as at midbodies in late cytokinesis. They find that Nup58 colocalizes and physically interacts with γ-tubulin and SAS-6 in mitosis. The authors further report that Nup58 is present in purified midbody fractions and colocalizes with KIF4 at intercellular bridges. Finally, depletion of Nup58 leads to centrosomal abnormalities and prolonged abscission timing. Based on these data, the authors conclude that Nup58 is important for mitosis, which provides a good starting point for a very interesting story.

Many of the conclusions, however, are not fully supported by the presented data. Throughout the manuscript the observations on Nup58 localization are inconsistent, possibly due to overexpression of Nup58 at unknown levels. Many experiments lack quantitative analysis, statistical testing, and need additional controls. The centrosome abnormalities and abscission delay upon Nup58 depletion suggest that Nup58 could have a role in mitosis, however, the claims that Nup58 is a regulator of midbody maintenance and microtubule dynamics are not supported by the data. Statements like "Nup58 functions to synchronize or accelerate late-stage midbody maturation processes" (line 225), or that Nup58 depletion results in "altered dynamics of microtubules" (line 81) or "increases disorder in central spindle microtubules" (line 275) are speculative and must be removed to avoid confusion. Overall, the manuscript in its current form is not suitable for publication unless the points outlined
below are addressed.

Major points

1. Given that Nup58 is a component of the nuclear pore complex, it is possible that Nup58 depletion causes pleiotropic perturbations stemming from defective nuclear transport, which may affect other proteins

required for mitosis and cytokinesis. After depletion of Nup58 for 3 days, cells could have accumulated a number of other defects that lead to centrosomal abnormalities and abscission delay. It will be important to confirm that these mitotic phenotypes are specific to Nup58 and not a secondary effect arising from other problems with nuclear transport. Authors should confirm that perturbation of other components of the NPC do not induce the same phenotypes that they claim to be specific for Nup58.

2. One of the major conclusions of the manuscript is the localization of Nup58 at centrosomes and midbodies. However, there are some discrepancies between Nup58 localization shown in the immunostainings compared with the Nup58-GFP construct. In the immunostainings the enrichment of Nup58 at centrosomes is very pronounced, while at the midbodies or at the nuclear rim it is only slightly brighter than the background staining. In the Nup58-GFP construct, on the other hand, the signal is high at midbodies, while the localization at centrosomes during metaphase and anaphase is barely visible (see Video 1). Another difference is the localization within the midbody, where endogenous Nup58 localizes as two bands (Fig 4c-f), while GFP-tagged Nup58 is in the central midbody bulge (Fig 3d). Nup58-GFP is expressed from a strong CMV promoter, which raises the concern that the overexpression might alter its dynamics and localization (overexpressed abscission components often localize to
the midbody center). Moreover, the cells shown in Video 2 and 3 exhibit extreme membrane blebbing, which might indicate toxicity of the overexpressed protein (or phototoxicity).
Authors should compare the levels of endogenous Nup58 with Nup58-GFP, and if needed express Nup58 at physiological levels using an endogenous promoter or CRISPR tagging. They should further confirm that expression of Nup58-GFP does not induce mitotic phenotypes.

3. The reduction of Nup58 levels following siRNA-mediated depletion is not very strong (Fig 5b), suggesting that the siRNA might not be the optimal choice. The authors should test other siRNAs to rule out off-target effects and validate the observed phenotypes with rescue experiments using an siRNA-resistant version of Nup58. It is concerning that even in control siRNA conditions 20% of cells have centrosome abnormalities (Fig 5d), which the authors need to address with additional controls.

4. Some experiments lack appropriate quantifications and statistical analysis. The quantifications in Fig 4c-d suggest that the images were saturated, since the fluorescence intensity does not reach a peak but is cut off around gray value ~60,000. The authors should use non-saturated images for this quantification. Moreover, the statistical testing described in line 204 is not presented in the manuscript: "Notably, Nup62 and SAS-6 levels were significantly reduced compared with mock-treated (control siRNA) cells (Figure 5b)."

5. In Fig 5d, the authors report a two-fold increase in 'centrosome abnormalities' upon Nup58-depletion compared to the control siRNA, however the type of these centrosome abnormalities is not defined (is it structural defects, altered centrosome numbers or both?). Moreover, the authors mention in line 211 that "down-regulation of Nup58 also induced a marked ~25% increase in the formation of monopolar and excessive centrosomes (co-staining with γ-tubulin, as a centrosome marker) (Figure 5d-e)", yet this quantification does not seem to be shown in any of the figure panels. Are these monopolar and supernumerary centrosomes included in the quantification in Fig 5d?

Minor points

1. Fig1b: Authors should clarify what is shown in the two bottom panels.

It looks like in the upper panel Nup58 is cytoplasmic whereas it is nuclear in the bottom panel. Are these images of the same cell taken at different planes? If so, this needs to be clarified; otherwise the difference in localization should be commented on.

2. Video 3: Please add a panel showing Nup58-GFP localization without the brightfield overlay.

3. Video 4 and Fig 5h: It looks like the cells are not aligned temporally (the control cell enters anaphase earlier than the Nup58-depleted cell). It is also easy to miss that the panels in Fig 5h have different time stamps, making it look like there is no difference between the conditions. Moreover, the movie of the control cell ends before the intercellular bridge is disconnected. The authors should show the full duration of the videos.

4. Fig 4c: The γ-tubulin signal looks strange and it is not clear where the midbody microtubules are. It would be helpful to label the microtubules to have a reference point, and use a different example for the γ-tubulin staining.

5. The language of the manuscript should be improved by removing typos (such as those in lines 46, 84, 112, 157 and 207) and fixing some mistakes, for example:
- Line 77: "In this report, we address whether Nup58 plays a role in regulating cell cycle gene expression". There is no data on expression of cell cycle genes in this manuscript.
- Line 276: "This may arise from the role of Nup58 in bundling and anchoring microtubules at the center of the midzone (45)." This reference seems inappropriate.
- Line 277 "Our finding that Nup58 depletion induces cells to be stuck in abscission for hours also raises the idea that Nup58 supports effectual abscission by stabilizing microtubules." This is confusing, since microtubules have to be disassembled to complete abscission.

If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES-LS) and American Journal Experts (http://bit.ly/AJE-LS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

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Response to reviewer_BMC

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HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id>
to Richard

Dear Prof Richard Wong,
I apologize for taking a longer time to finish the response. Please find attached the file of reviewer response, main figures, su
submission to BMC.
Thank you very much for your support.
Kind regards,
Hartono

Supplementary Pig_MBC_Revision_Hartono.rar
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4 Attachments • Scanned by Gmail

Reviewer respons.
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HARTONO UNM <hartono@unm.ac.id>

Re: R1 Nup58 MS

4 messages

rwong@staff.kanazawa-u.ac.jp <rwong@staff.kanazawa-u.ac.jp> 17 June 2019 at 17:00
To: "HARTONO, S.Si, S.Pd, M.Biotech UNM" <hartono@unm.ac.id>

Dear Hartono,

Please double check the MS carefully.

Have I added all your revision points ?

If no problem, I shall send to company for proofing.

Best,
Richard

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272K

HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id> 17 June 2019 at 17:16
To: Richard Wong <rwong@staff.kanazawa-u.ac.jp>

Dear Prof. Richard Wong,

I received the Manuscript. Now, I am checking the MS.

Thank you very much.

Kind regards,

Hartono

[Quoted text hidden]

HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id> 18 June 2019 at 03:58
To: Yenni Yusuf <yenniyusuf@icloud.com>

[Quoted text hidden]

3 attachments

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HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id> 18 June 2019 at 07:57
To: Richard Wong <rwong@staff.kanazawa-u.ac.jp>

Dear Prof Richard Wong,

Please find attached the revised manuscript. There is only a few things to be changed
I have edited some points, and highlighted them with yellow mark.

Thank you in advance for your support.

Kind regards,

Hartono

[Quoted text hidden]

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Inbox ×

Richard Wong <rwongkanazawa@gmail.com>
to me

Hi Hartono,

Here is the final proof, with revised text and new figures.

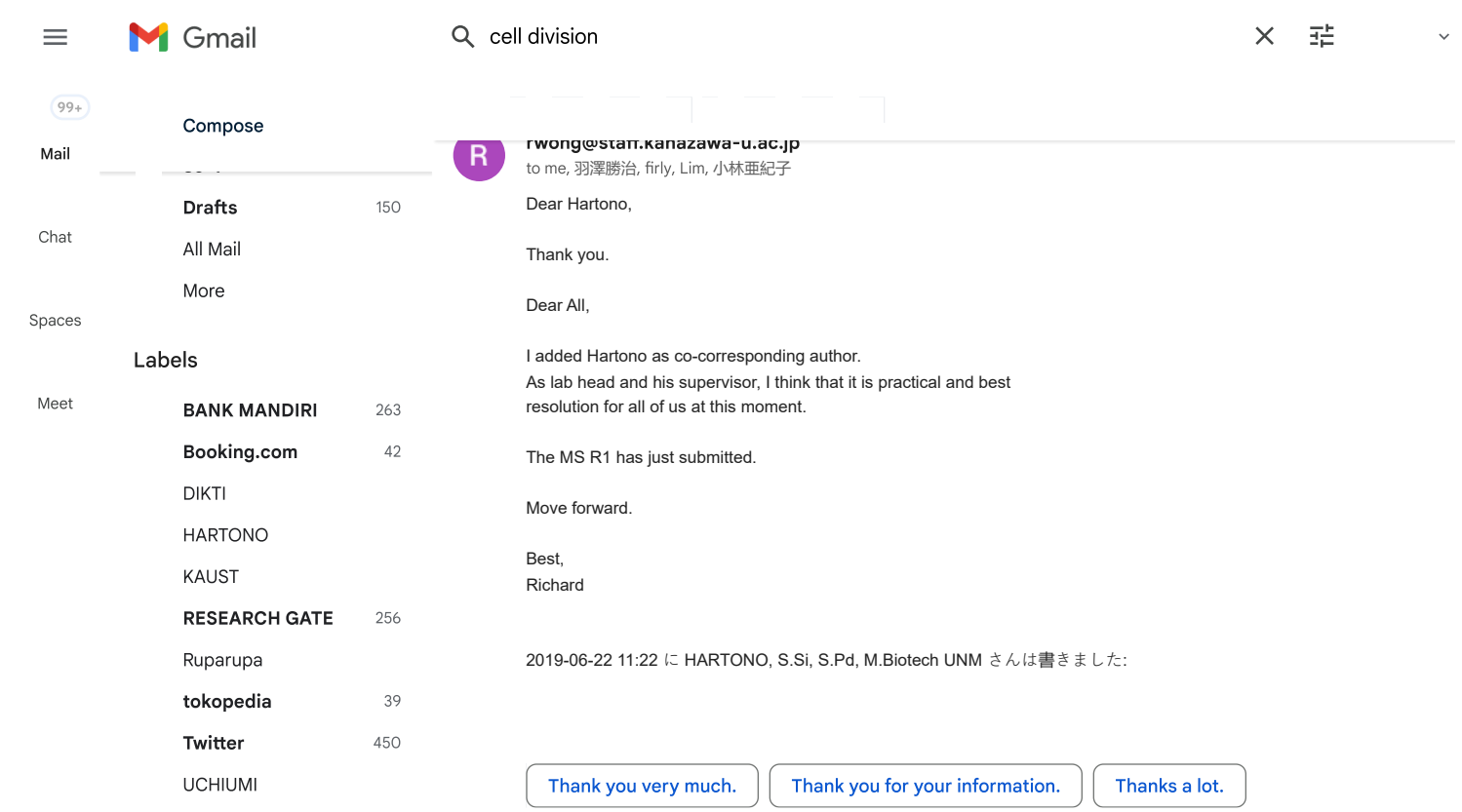
Please double check really carefully.

Please also send me the whole set of supp figures.

Best,
Richard

One attachment • Scanned by Gmail

20190621_Nup58_.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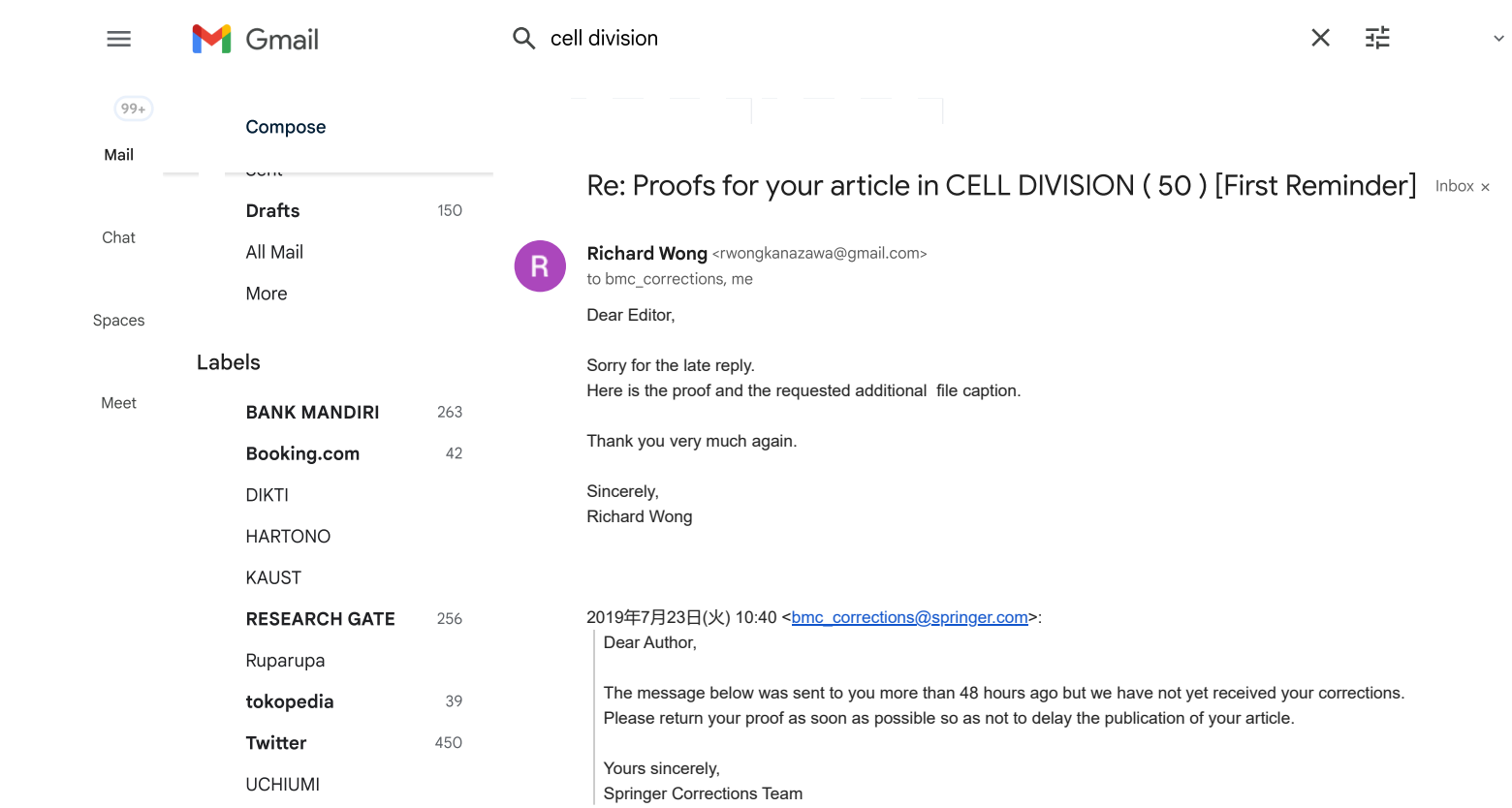
to me, 羽澤勝治, firly, Lim, 小林亜紀子
Dear Hartono,
Thank you.
Dear All,
I added Hartono as co-corresponding author.
As lab head and his supervisor, I think that it is practical and best resolution for all of us at this moment.
The MS R1 has just submitted.
Move forward.
Best,
Richard
2019-06-22 11:22 に HARTONO, S.Si, S.Pd, M.Biotech UNM さんは書きました:
Thank you very much.
Thank you for your information.
Thanks a lot.



Re: Proofs for your article in CELL DIVISION (50) [First Reminder]

Inbox ×

Richard Wong <rwongkanazawa@gmail.com>
to bmc_corrections, me

Dear Editor,

Sorry for the late reply.
Here is the proof and the requested additional file caption.

Thank you very much again.

Sincerely,
Richard Wong

2019年7月23日(火) 10:40 <bmc_corrections@springer.com>:

> Dear Author,
>
> The message below was sent to you more than 48 hours ago but we have not yet received your corrections.
> Please return your proof as soon as possible so as not to delay the publication of your article.
>
> Yours sincerely,
> Springer Corrections Team

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HARTONO UNM <hartono@unm.ac.id>

Really good news from BMC Cell Division

3 messages

rwong@staff.kanazawa-u.ac.jp <rwong@staff.kanazawa-u.ac.jp> 3 July 2019 at 11:21
To: "HARTONO, S.Si, S.Pd, M.Biotech UNM" <hartono@unm.ac.id>
Cc: 羽澤勝治 <masaharu.akj@gmail.com>, firly rahmah <firlyrahmah@gmail.com>, Lim keesiang <xiscolim@gmail.com>, 小林亜紀子 <akoba@staff.kanazawa-u.ac.jp>

Dear All,

Really good news arrived.
Congratulations to Hartono!

Prepare well the oral thesis defense, you can graduate on-time.

Keep it in secret till the lab meeting, I shall open this to all.

Best,
Richard

CDIV-D-19-00011R1
Nucleoporin Nup58 localizes to centrosomes and mid-bodies during mitosis
Hartono Hartono; Masaharu Hazawa; Kee Siang Lim; Firli R.P. Dewi; Akiko Kobayashi; Richard Wong
Cell Division

Dear Prof. Wong,

I am pleased to inform you that your manuscript "Nucleoporin Nup58 localizes to centrosomes and mid-bodies during mitosis" (CDIV-D-19-00011R1) has been accepted for publication in Cell Division.

Before publication, our production team will check the format of your manuscript to ensure that it conforms to the standards of the journal. They will be in touch shortly to request any necessary changes, or to confirm that none are needed.

Any final comments from our reviewers or editors can be found, below. Please quote your manuscript number, CDIV-D-19-00011R1, when inquiring about this submission.

We look forward to publishing your manuscript and I do hope you will consider Cell Division again in the future.

Best wishes,

Philipp Kaldis, PhD
Cell Division

HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id> 3 July 2019 at 11:54
To: yenni yusuf <yenni.ys@gmail.com>

[Quoted text hidden]

HARTONO, S.Si, S.Pd, M.Biotech UNM <hartono@unm.ac.id> 3 July 2019 at 12:14
To: Richard Wong <rwong@staff.kanazawa-u.ac.jp>
Cc: 羽澤勝治 <masaharu.akj@gmail.com>, firly rahmah <firlyrahmah@gmail.com>, Lim keesiang <xiscolim@gmail.com>, 小林亜紀子 <akoba@staff.kanazawa-u.ac.jp>

Dear All,

I would like to thank for all your support and suggestion. Especially for Prof. Richard Wong, my sincere appreciation for his continuous support and help.

Without it, this publication was impossible.

Again, thank you very much.

Kind regards,

Hartono

[Quoted text hidden]