The PRDM14-CtBP1/2-PRC2 complex regulates transcriptional repression during the transition from primed to naive pluripotency

Maiko Yamamoto, Yoshiaki Suwa, Kohta Sugiyama, Naoki Okashita, Naoki Tani, Masanori Kawaguchi, Kazumi Matsubara, Akira Nakamura and Yoshiyuki Seki
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Original submission

First decision letter

MS ID#: JOCES/2019/240176

MS TITLE: PRDM14-CtBP1/2-PRC2 complex regulates transcriptional repression during transition from primed to naive pluripotency

AUTHORS: Maiko Yamamoto, Yoshiaki Suwa, Kohta Sugiyama, Naoki Okashita, Naoki Tani, Masanori Kawaguchi, Kazumi Matsubara, Akira Nakamura, and Yoshiyuki Seki
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Synopsis: In this manuscript by Yamamoto et al, the authors describe a mechanism for the PRDM14-mediated regulation of transcriptional repression in mouse ES cells (mESCs). PRDM14 is a DNA-binding transcription factor with a pivotal role in the regulation of mESC pluripotency, determined through several gain- and loss-of-function studies described in prior publications. Different biochemical approaches have implicated the transcriptional co-repressor CBFA2T2 and PRC2-subunit Suz12 in mediating the transcriptional repression exerted upon PRDM14 binding a specific genomic locus.

Here, the authors focus on elucidating the mechanism through which PRDM14 modulates target gene expression by using a doxycycline-inducible system for the expression of full-length PRDM14 or mutants lacking either the N-terminus or the PR domain in prdm14-deficient mESCs. By using affinity purification mass spectrometry analyses of proteins that co-immunoprecipitated with FLAG-tagged PRDM14 variants, the authors identified an important role for the N-terminus of PRDM14 as a protein-protein interaction interface or scaffold. In agreement with prior studies, the authors detected CBFA2T2 in their PRDM14 co-IPs as well as components of the NODE, esBAF and CRL4 complexes and CtBP2.

Although they were unable to detect PRC2 complex components described in a prior BioID study, their detection of CtBP2 prompted them to follow up on it based on its reported role in the recruitment of PRC2 components. By generating CBFA2T2 knockout mESCs, the authors found that the co-IP of CtBP2 and PRDM14 required CBFA2T2, suggesting that CBFA2T2 serves as a bridge between PRDM14 and CtBP2. ChIP-qPCR experiments revealed that recruitment of CtBP2-HDAC1 to repressed PRDM14 targets required PRDM14 recruitment and was not simply coincidental. By contrast genes upregulated by PRDM14 recruitment were found to be associated with increased TET1 enrichment and decreased CtBP2 binding in publicly available ChIP-seq datasets.

By employing Suz12 knockout mESCs, the authors further elaborate the mechanism of PRDM14-mediated gene repression via CtBP2, providing data supporting a role for Suz12 in this process and in the negative-feedback regulation of PRDM14 on its own expression. They also report that Suz12 plays a role in the PRDM14-promoted conversion of mEpiLCs to mESCs, cooperatively activating pluripotency-associated genes and repressing differentiation markers.

Knockout experiments targeting Ctbp1/2 genes revealed a partial requirement for these genes in PRDM14-mediated transcriptional repression, with an absolute requirement for CtBP1/2 in the repression of Dnmt3b transcription by PRDM14. Intriguingly, whereas PRDM14-null mESCs can be propagated in LIF+2i conditions as colonies in a naive pluripotent state, Ctbp1/2-null cells cannot, highlighting PRDM14-independent functions of Ctbp1/2 in mESC pluripotency.

Critical Overview: Overall, this is a very nice study that provides new information about the mechanisms through which PRDM14 regulates target genes in pluripotent mouse cells. The authors' findings provide a plausible explanation for the discrepancies in the literature regarding the role of PRC2 complexes in mediating at least some repressive functions of PRDM14, with PRDM14 recruiting PRC2 via CtBP1/2 at repressive PRDM14 targets. Although there is much that remains to be resolved, this study provides important new information that will be of interest to those interested in transcriptional regulation by PRDM14 in pluripotent cells.

Comments for the author

Major concerns:
As mouse embryonic stem cells in LIF + serum conditions are not homogeneous, it would be informative to undertake re-ChIP analyses for at least a subset of the genomic targets that are proposed to be co-occupied by PRDM14 and CtBP2 (Fig. 2D), PRDM14 and TET1/TET2 (Fig. 2F).
In Fig. 4B the authors present morphological data for their Ctbp2 and Ctbp1/2 DKO mESCs. This data would be greatly improved by also providing immunofluorescent/immunohistochemical staining for pluripotent stem cell markers and alkaline-phosphatase staining (as done for the Suz12 KOs in Fig. 5). For quantitative purposes, western blots for pluripotent stem cell markers (e.g. Oct4

Sox2 and Nanog) would also be informative. The study relies heavily on transcript levels, which could be misleading and should be supplemented with protein data.
The morphological data in Figs. 6A, 6E, 7A would also be more informative if supplemented with (minimally) AP staining as well as higher-magnification images (insets).

Minor concerns:

1. The last sentence on page 16 is confusing as written. The first phrase would be easier to interpret if written as “As observed in Suz12 KO ESCs”.

Reviewer 2

Advance summary and potential significance to field

The PR domain-containing protein, PRDM14 is a pivotal molecule in pluripotency and the germline. PRDM14 is a sequence-specific transcriptional regulator that can activate and repress transcription dependent on target genes. This transcriptional dichotomy is evident in epiblast-like cells where PRDM14 activates pluripotency genes such as Klf2 and Tc11 and represses epiblast markers such as Otx2, Fgf5 and Dnmt3b. PRDM14 activates pluripotency genes through OCT4 recruitment and TET-mediated DNA demethylation, however, the molecular mechanism by which PRDM14 represses is not known. This is an important question in the field and here Yamamoto, et al address the mechanism for PRDM14 repression during the transition from primed to naïve pluripotency. The authors show that the N-terminal domain of PRDM14 is necessary to acquire and maintain pluripotency. This domain has not been shown to have a defined function. Yamamoto, et al engineer a doxycycline-inducible expression system to generate PRDM14 full length protein and PRDM14 mutants lacking N-terminal- or the PR domain- in Prdm14-deficient mouse embryonic stem cells (ESCs).
Ablation of the N-terminal domain of PRDM14 abolished the maintenance of ESC pluripotency whereas the PR domain mutation partially supported the self-renewal activity of the Prdm14-deficient ESCs. A mass spec analysis reveals a PRDM14-containing complex identifies N-terminal partnering proteins that bind the N-terminal domain. The authors then examine knockouts of these interacting proteins (Ctbp1/2, Suz12 and Cbfa2t2) to show that CTBP1/2 complexes with PRDM14-CBFA2T2 with PRC2 at repressed genes in ESCs.
Overall, I find this work is impactful to the field and the authors have gone to great strides to present a complete story. There are several items that would enhance this manuscript prior to publication.

Comments for the author

Figure 1A I would show a scheme of the PRDM14 domains (From S1B) above the silver stain immunoprecipitants. The authors should indicate the proteins on this silver stain panel. S1H should be moved to the primary data in Figure 1. The peptide counts could be moved to the supplemental data. Supplemental S1 G is very hard to see. CBFA2Ts was detected in the full length PRDM14 complex but absent from the N- and PR- mutant PRDM14 complexes. It is not indicated whether the co-immunoprecipitation (coIP) in panel D and E are from primed or naïve cells. Are these whole cell lysates? This needs to be stated in the legend. The authors generate Cbfa2t2 KO ESCs carrying a doxycycline-inducible Prdm14. In the absence of Cbfa2t2 Flag-PRDM14 failed to be coIPed with CTBP2 (which can recruit the PRC2 complex to gene targets) suggesting that the PRDM14-CTBP2 interaction is CBFA2T2-dependent. The coIP Western anti-Flag and IP CTBP2 in panel E needs to be repeated for publication.

Figure 2 interrogates the ability of PRDM14 and CtBP2 at ESC gene targets. The legend does not describe Figure 2G (text describes PRMD14 interaction with TET2 but it is not indicated which protein has the appropriate epitope tag). Can the authors demonstrate endogenous interaction?

Figure 5C. Please provide clearer images for publication.

First revision

Author response to reviewers' comments

We would like to thank all 2 referees for their critical comments to improve our study for the publication.

Reviewer 1 Advance summary and potential significance to field
Synopsis: In this manuscript by Yamamoto et al, the authors describe a mechanism for the PRDM14-mediated regulation of transcriptional repression in mouse ES cells (mESCs). PRDM14 is a DNA-binding transcription factor with a pivotal role in the regulation of mESC pluripotency, determined through several gain- and loss-of-function studies described in prior publications. Different biochemical approaches have implicated the transcriptional co-repressor CBFA2T2 and PRC2-subunit Suz12 in mediating the transcriptional repression exerted upon PRDM14 binding a specific genomic locus.

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Although they were unable to detect PRC2 complex components described in a prior BioID study, their detection of CtBP2 prompted them to follow up on it based on its reported role in the recruitment of PRC2 components. By generating CBFA2T2 knockout mESCs, the authors found that the co-IP of CtBP2 and PRDM14 required CBFA2T2, suggesting that CBFA2T2 serves as a bridge between PRDM14 and CtBP2. ChIP-qPCR experiments revealed that recruitment of CtBP2-HDAC1 to repressed PRDM14 targets required PRDM14 recruitment and was not simply coincidental. By contrast genes upregulated by PRDM14 recruitment were found to be associated with increased TET1 enrichment and decreased CtBP2 binding in publicly available ChIP-seq datasets.

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We are very grateful for the reviewer's positive comments and suggestion to improve our study. We have added the new data and revised the manuscript based on the reviewer's comments. Our point-by point responses to the reviewer's comments are as follows:

Reviewer 1 Comments for the author
Major concerns:

Comment 1.
As mouse embryonic stem cells in LIF + serum conditions are not homogeneous, it would be informative to undertake re-ChIP analyses for at least a subset of the genomic targets that are proposed to be co-occupied by PRDM14 and CtBP2 (Fig. 2D), PRDM14 and TET1/TET2 (Fig. 2F).

Response 1.
We completely agree with the comments. We have performed re-ChIP analysis with anti-FLAG (FLAG-PRDM14) followed by anti-CtBP2 or TET1 antibody, and found the colocalization of PRDM14 with CtBP2 or TET1 at target genes (Figs. 2H, I).

Comment 2
In Fig. 4B the authors present morphological data for their Ctbp2 and Ctbp1/2 DKO mESCs. This data would be greatly improved by also providing immunofluorescent/immunohistochemical staining for pluripotent stem cell markers and alkaline-phosphatase staining (as done for the Suz12 KOs in Fig. 5).

Response 2.
We agree the reviewer's comment and performed immunofluorescence with anti-OCT4 and NANOG in WT and Ctbp1/2 DKO ESCs (Fig. 4C). We also performed alkaline phosphatase staining in WT and Ctbp1/2 DKO ESCs under serum plus LIF and 2i plus LIF condition (Fig. 6G).

Comment 3.
For quantitative purposes, western blots for pluripotent stem cell markers (e.g. Oct4, Sox2 and Nanog) would also be informative. The study relies heavily on transcript levels, which could be misleading and should be supplemented with protein data.

Response 3.
We are very grateful for the critical comments. According to the reviewer's comments, we have performed western blotting with anti-OCT4 and NANOG in WT and Ctbp1/2 DKO ESCs (Fig. 4D).

Comment 4.
The morphological data in Figs. 6A, 6E, 7A would also be more informative if supplemented with (minimally) AP staining as well as higher-magnification images (insets).

Response 4.
As requested, we have changed to higher-magnification images (Figs. 6A,F and Fig. 7A), and added AP staining of WT, Suz12 KO and Ctbp1/2 DKO ESCs under serum plus LIF and 2i plus LIF.

Minor concerns:

Comment 5.
The last sentence on page 16 is confusing as written. The first phrase would be easier to interpret if written as "As observed in Suz12 KO ESCs".

Response 5.
We agree with this point. We have changed to "As observed in Suz12 KO ESCs,…" (Page 20, Line 12)

Reviewer 2 Advance summary and potential significance to field
The PR domain-containing protein, PRDM14 is a pivotal molecule in pluripotency and the germline. PRDM14 is a sequence-specific transcriptional regulator that can activate and repress transcription dependent on target genes. This transcriptional dichotomy is evident in epiblast-like cells where PRDM14 activates pluripotency genes such as Klf2 and Tc11 and represses epiblast markers such as Otx2, Fgf5 and Dnmt3b. PRDM14 activates pluripotency genes through OCT4 recruitment and TET-mediated DNA demethylation, however, the molecular mechanism by which PRDM14 represses is not known. This is an important question in the field and here Yamamoto, et al address the mechanism for PRDM14 repression during the transition from primed to naïve pluripotency. The authors show that the N-terminal domain of PRDM14 is necessary to acquire and maintain

pluripotency. This domain has not been shown to have a defined function. Yamamoto, et al engineer a doxycycline-inducible expression system to generate PRDM14 full length protein and PRDM14 mutants lacking N-terminal- or the PR domain- in Prdm14-deficient mouse embryonic stem cells (ESCs). Ablation of the N-terminal domain of PRDM14 abolished the maintenance of ESC pluripotency whereas the PR domain mutation partially supported the self-renewal activity of the Prdm14-deficient ESCs. A mass spec analysis reveals a PRDM14-containing complex identifies N-terminal partnering proteins that bind the N-terminal domain. The authors then examine knockouts of these interacting proteins (Ctbp1/2, Suz12 and Cbfa2t2) to show that CTBP1/2 complexes with PRDM14-CBFA2T2 with PRC2 at repressed genes in ESCs.
Overall, I find this work is impactful to the field and the authors have gone to great strides to present a complete story. There are several items that would enhance this manuscript prior to publication.

We are very grateful for the reviewer's positive comments and suggestion to improve our study. We have added the new data and revised the manuscript based on the reviewer's comments. Our point-by point responses to the reviewer's comments are as follows:

Reviewer 2 Comments for the author
Comment 1.
Figure 1A I would show a scheme of the PRDM14 domains (From S1B) above the silver stain immunoprecipitants.

Response 1.
We have moved a scheme of the PRDM14 domains to the Fig. 1A

Comment 2.
The authors should indicate the proteins on this silver stain panel.

Response 2.
We have performed shotgun-LC/MS not cut the band of silver staining. Therefore, we could not indicate the proteins and added the explanation in the results section (Page 9, Line 18)

Comment 3.
S1H should be moved to the primary data in Figure 1.

Response 3.
We have moved the data of EpiLCs with PRDM14 to Fig. 1C.

Comment 4.
The peptide counts could be moved to the supplemental data.

Response 4.
Thank you for the suggestion. We would like to emphasize that PRDM14 form several distinct complexes, we did not have moved to the supplemental data.

Comment 5.
Supplemental S1 G is very hard to see.

Response 5.
According to the reviewer's comment, we have changed to the high magnification image (Fig. 1 C)

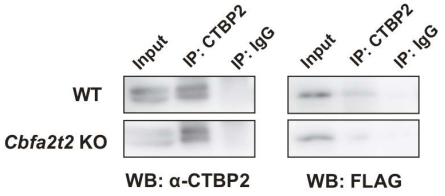
Comment 5.
CBFA2Ts was detected in the full length PRDM14 complex but absent from the N- and PR- mutant PRDM14 complexes. It is not indicated whether the coimmunoprecipitation (coIP) in panel D and E are from primed or naïve cells. Are these whole cell lysates? This needs to be stated in the legend.

Response 5.
We are grateful for the pointing out of the lack of our explanation for the culture condition and experimental condition. We have described about culture condition and experimental condition (we

used the whole cell lysate for IP experiment) in the results section (Page 10, Line 2, 3) and in the figure legend.

Comment 6.
The authors generate Cbfa2t2 KO ESCs carrying a doxycycline-inducible Prdm14. In the absence of Cbfa2t2, Flag-PRDM14 failed to be coIPed with CTBP2 (which can recruit the PRC2 complex to gene targets) suggesting that the PRDM14-CTBP2 interaction is CBFA2T2-dependent. The coIP Western anti-Flag and IP CTBP2 in panel E needs to be repeated for publication.

Response 6.
We completely agree with the authors comments and performed immunoprecipitation with anti-CtBP2 and followed by WB with anti-FLAG in wild type and Cbfa2t2 KO ESCs (Figure for reviewer).



We have obtained same results that the interaction of PRDM14 with CtBP2 is impaired by the loss of Cbfa2t2. We have detected the weak interaction between PRDM14 and CtBP2 in the absence of Cbfa2t2. Mouse genome has two paralogues of CBFA2T2, CBFA2T1 and CBFA2T3. Cbfat2t2 is highest expressed among three paralogues in mouse embryonic stem cells, but Cbfat2t1/3 are slightly expressed in mESCs. Therefore, CBFA2T1/3 might be involved in the weak interaction of PRDM14 with CtBP2 in the absence of Cbfa2t2. Supporting this possibility, our LC/MS/MS data showed that the low counts of peptides derived from CBFA2T1 was included in PRDM14 complex.

Comment 7.
Figure 2 interrogates the ability of PRDM14 and CtBP2 at ESC gene targets. The legend does not describe Figure 2G (text describes PRMD14 interaction with TET2 but it is not indicated which protein has the appropriate epitope tag). Can the authors demonstrate endogenous interaction?

Response 7.
We are very grateful for the critical comments. This data is the interaction of recombinant of FLAG-PRDM14 with the recombinant HA-TET2 in the cell-free system. We have added the taq information in the Figure and results section (Page 13, Line 15). We also have added the detailed explanation in the legends (Page 41, Line 8)

Comment 8.
Figure 5C. Please provide clearer images for publication.

Response 8.
According to the reviewer's comment, we have changed to the high-magnification image of AP staining (Fig. 5C).

Second decision letter

MS ID#: JOCES/2019/240176

MS TITLE: PRDM14-CtBP1/2-PRC2 complex regulates transcriptional repression during transition from primed to naive pluripotency

AUTHORS: Maiko Yamamoto, Yoshiaki Suwa, Kohta Sugiyama, Naoki Okashita, Naoki Tani, Masanori Kawaguchi, Kazumi Matsubara, Akira Nakamura, and Yoshiyuki Seki
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers recognize that most of their initial criticisms have been addressed in your revised manuscript. However, reviewer #1 still raised issues that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors provide new insights into how the sequence-specific transcription factor PRDM14 regulates target gene transcription during the transition from primed to naive pluripotency in the mouse. The authors employ proteomics and a series of experiments with knockout cell lines to dissect the complexes employed by PRDM14 to differentially regulate specific target genes. The authors' findings provide a plausible explanation for the discrepancies in the literature regarding the role of PRC2 complexes in mediating at least some repressive functions of PRDM14, with PRDM14 recruiting PRC2 via CtBP1/2 at repressive PRDM14 targets.
Although there is much that remains to be resolved, this study provides important new information that will be of interest to those interested in transcriptional regulation by PRDM14 in pluripotent cells.

Comments for the author

Overview:

The authors have done a commendable job of responding to previous reviewer concerns, and I have only minor concerns that remain to be addressed.

Minor Concerns:

1. There are a few typographical and grammatical errors that reduced the clarity of the writing, which are listed below:

Line 33: "at the target gene" should be "at target genes".

Line 41: This sentence was difficult to follow, and I recommend that the section "expression is maintained from the inner cell mass (ICM) in the blastocyst to the" be replaced by "expression in inner cell mass (ICM) cells of the blastocyst is maintained in".

Line 44: “Somatic lineage maintains” would be improved by changing to “Somatic cells maintain.

Line 45: “are specified from most proximal epiblast cells associated” would be improved by changing to “specified from the most proximal epiblast cells are associated”.

Line 58: “shuts off the autocrine of fibroblast” should be “shuts off autocrine fibroblast”.

Line 63: “component” should be “components”.

Line 112: “does not have discernable functional domain” should be “does not have a discernible functional domain”.

Lines 238-39: “heterogenous” should be “heterogeneous”, and “homogenous” should be “homogeneous”.

Line 241: “transcription factor” should be “transcription factors”.

Line 325: “Prdm14 was activated in most proximal epiblast cells” should be “Prdm14 is activated in the most proximal epiblast cells”.

Line 379: “genes recognition” should be “gene regulation”.

Line 392: “St. Lousi” should be “St. Louis”.

Line 468: “immnuprecipiation” should be “immunoprecipitation”.

2. One line 61, Gsk3β, should be replaced by GSK-3 (no β). Embryonic stem cells express GSK-3α and GSK-3β, which are essentially identical in their kinase domains. Thus, the GSK-3 inhibitor (CHIR99021) is not specific to GSK-3β, as it inhibits both GSK-3α and GSK-3β. The functional redundancy of the two GSK-3 gene products has been described in a mouse embryonic stem cell model (Doble et al., Developmental Cell, 2007 - https://doi.org/10.1016/j.devcel.2007.04.001).

3. For figures 1D and suppl. 1C, no descriptions of the statistical analyses used to generate the graphs are provided.

4. For the bar graphs presented in Figure 2, I believe it would be more appropriate to present the standard deviations instead of standard errors, as only technical duplicates were analyzed.

5. The “Immunofluorescence analysis” component of the materials and methods section should provide the details of the secondary fluorophore-conjugated antibodies used.

6. The authors should ensure that they properly acknowledge the PI who provided Addgene plasmid 42230, as indicated on the addgene website under “How to cite this plasmid”. http://www.addgene.org/42230/

Reviewer 2

Advance summary and potential significance to field

The authors have done an excellent job in addressing questions and performing additional experiments. This manuscript by Yamamoto et al describes a mechanism for PRDM14-mediated regulation in stem cells and is important to the pluripotent field. The manuscript is ready for publication.

Comments for the author

Excellent work.

Second revision

Author response to reviewers' comments

We are grateful for pointing out of a few typographical and grammatical errors to improve our manuscript. We have revised the sentence and added information about methods.

Third decision letter

MS ID#: JOCES/2019/240176

MS TITLE: PRDM14-CtBP1/2-PRC2 complex regulates transcriptional repression during transition from primed to naive pluripotency

AUTHORS: Maiko Yamamoto, Yoshiaki Suwa, Kohta Sugiyama, Naoki Okashita, Naoki Tani, Masanori Kawaguchi, Kazumi Matsubara, Akira Nakamura, and Yoshiyuki Seki
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.