Frizzled3 inhibits Vangl2-Prickle3 association to establish planar cell polarity in the vertebrate neural plate

Ilya Chuykin, Keiji Itoh, Kyeongmi Kim and Sergei Y. Sokol
DOI: 10.1242/jcs.258864

Editor: Guangshuo Ou

Review timeline
Original submission: 4 May 2021
Editorial decision: 21 July 2021
First revision received: 10 September 2021
Editorial decision: 19 October 2021
Second revision received: 21 October 2021
Accepted: 9 November 2021

Original submission

First decision letter

MS ID#: JOCES/2021/258864

MS TITLE: Frizzled3 inhibits Vangl2-Prickle3 association to establish planar cell polarity in the vertebrate neural plate

AUTHORS: Ilya Chuykin, Kyeongmi Kim, and Sergei Sokol
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.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. 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

This manuscript reports a role of Fz3 for the establishment of PCP in the neural plate of Xenopus embryos.
The authors showed that Fz3 regulates the interaction between Vangl2 and Pk3 through Vangl2 phosphorylation on T76 and T78. Overexpression of Fz3 suppresses, whereas knockdown of Fz3 enhances Vangl2 and Pk3 association. A nonphosphorylatable form of Vangl2 fails to polarize with Pk3 in neural plate cells. Based on these observations, the authors postulate that Fz3 regulates the formation and anterior localization of Vangl2-Pk3 complex by inducing the phosphorylation of T76 and T78 in Vangl2. Results from this study raise the possibility that Fz3-mediated Vangl2 phosphorylation at specific sites may participate in the establishment of epithelial polarity. They could help to understand the regulation of neural plate PCP and would be of potential interest to the fields of Wnt signaling as well as cell and developmental biology.

Comments for the author

The requirement of Fz3 for the formation and polarization of Vangl2-Pk3 complex in the neural plate was not convincingly demonstrated in the manuscript. A number of issues need to be addressed as listed below.

Major points:
1. Consistent with previous observations from the authors, Figure 1 nicely shows anterior accumulation of Vang2 in CoMO-injected neural plate cells. Knockdown of Fz3 perturbs the anterior accumulation of Vangl2 however, this also disrupts Vangl2 membrane localization. Thus it is not clear whether the absence of anterior Vangl2 in Fz3 morphant cells is simply due to the absence of Vangl2 membrane localization. This point needs to be clarified. In addition, it may be also necessary to examine how Fz3 overexpression affects the anterior accumulation or membrane localization of Vangl2.

2. What is the localization of Fz3 in neural plate cells? The Fz3 antibody seems to work in western blot, the authors may also try to see if it works in IF. Otherwise, the localization of exogenous Fz3 may be examined.

3. Although biochemical analyses show that knockdown of Fz3 or expression of nonphosphorylatable Vangl2 enhances Vangl2-Pk3 complex formation, the influence of Fz3 on Vangl2-Pk3 localization in the neural plate is also unclear. It may be necessary to analyze how Vangl2-Pk3 complex is localized in neural plate cells following Fz3 overexpression and knockdown.

4. Page 8: “Vangl2 was compared with Vangl2-7A, which does not dissociate from the Pk3 protein in response to Fz3”. This is clearly shown by co-immunoprecipitation assay (Figure 6C). However, data from in vivo experiments are not fully consistent with the results from biochemical analyses. While Vangl2 and Pk3 showed exclusive complex formation and co-localization in the neural plate (Figure 7A,A’), co-expression of the nonphosphorylatable Vangl2-7A with Pk3 not only disrupted anterior accumulation but also caused less co-localization (Figure 7B,B’), suggesting reduced association.

5. In the model presented in Figure 7D, it is not easy to understand the way by which posteriorly localized Fz3 inhibits Vangl2-Pk3 complex formation and anterior localization. How does negatively regulatory feedback between Fz3 and Vangl2 lead to their segregation to opposite cell sides? This is not adequately discussed in the legend or in main text.

Minor points:
1. Pages 5-6: “significant lower amounts of Vangl2 coprecipitated with Pk3 from Fz3-expressing explants”.
Figure 3B needs quantification given that an important conclusion of this study is the inhibitory role of Fz3 on the association of Vangl2 and Pk3 in Xenopus embryos.
2. The anterior localization of Vangl2 with respect to neural plate convergence and extension may be discussed further.

Reviewer 2

Advance summary and potential significance to field

The authors use proximity labeling and covalent crosslinking to show that the planar cell polarity proteins Vangl2 and Pk3 interact and that this interaction is disrupted by Fz3. Fz3 also disrupts the asymmetric distribution Vangl2 in Xenopus neural ectoderm. Their conclusions are supported by overexpression and morpholino depletion of Fz3. Fz3 overexpression also decreases the electrophoretic mobility of Vangl2, which they hypothesize (reasonably) is due to phosphorylation induced by Fz3; site directed mutagenesis of Vangl2 identifies residues that are necessary for phosphorylation of Vangl2 and disruption of the Vangl2/Pk3 complex. Overall, this work is interesting and moves the PCP field forward by defining a link between frizzled function and Vangl2/Prickle3 interaction in the developing embryo. There are some limitations to their interpretation that should be addressed by changes to the text but otherwise this work is a valuable contribution to the field.

Comments for the author

1. My main concern about their interpretation is the claim that T76 and T78 in Vangl2 are sites of Fz3-induced phosphorylation. While this is a reasonable hypothesis, they have not shown this. The T76/T78AA mutations may affect phosphorylation at other sites without being the primary site of Fz3-induced phosphorylation. Their own data show that mutation of serines 82 and 84 impairs phosphorylation at other sites (threonines, based on phospho-threonine immunoblotting (Fig. 5B)), clearly demonstrating that a mutation at one site can affect phosphorylation at other sites. They have shown that T76 and T78 are necessary, not that they are sites of phosphorylation, and certainly not that they are the primary sites of Fz3-induced phosphorylation. This could be addressed by changes to the text to remove this claim throughout the manuscript.

2. If “Fz3 inhibits Vangl2-Pk3 complex formation by triggering Vangl2 phosphorylation,” then why doesn’t Fz3 knockdown (by morpholino) reduce Vangl2 phosphorylation (Fig. 4B)? This can be addressed in the text and does not require new experiments.

3. Their interpretation of the 7A and 5A mutations in cluster II is unclear.
Perhaps this was a typographical error: “The association of Pk3 with Vangl2-7A was completely resistant to Fz3, whereas the complex containing Vangl2-5A was partially sensitive (Fig. 6A, C). These experiments indicate that the T76T78 phosphorylation is necessary for the inhibitory effect of Fz3 on PCP complex formation.” If the 5A mutations have a partial effect, then don’t they mean that both T76/T78 and the serines in cluster II contribute? Or do they interpret the small effect of the cluster II mutations to mean their contribution is minimal and should be ignored? This should be clarified, as the implication as written now is that the cluster II mutations have no role.

4. As the Fz3 morpholino has a strong effect on Vangl2 localization, the authors should comment on the morphological phenotype of morpholino injected embryos.
Furthermore, they note that nonphosphorylatable Vangl2 disrupts neural tube closure. If this is a real effect and not an artifact, they should show these results. If they have concern that the effect is nonspecific, then this claim should be removed from the text.

First revision

Author response to reviewers' comments

Responses to reviewers

We thank both reviewers for the constructive comments that helped us improve the manuscript.

Reviewer 1 Advance Summary and Potential Significance to Field:
This manuscript reports a role of Fz3 for the establishment of PCP in the neural plate of Xenopus embryos.
The authors showed that Fz3 regulates the interaction between Vangl2 and Pk3 through Vangl2 phosphorylation on T76 and T78. Overexpression of Fz3 suppresses, whereas knockdown of Fz3 enhances, Vangl2 and Pk3 association. A nonphosphorylatable form of Vangl2 fails to polarize with Pk3 in neural plate cells. Based on these observations, the authors postulate that Fz3 regulates the formation and anterior localization of Vangl2-Pk3 complex by inducing the phosphorylation of T76 and T78 in Vangl2. Results from this study raise the possibility that Fz3-mediated Vangl2 phosphorylation at specific sites may participate in the establishment of epithelial polarity. They could help to understand the regulation of neural plate PCP and would be of potential interest to the fields of Wnt signaling as well as cell and developmental biology.

Reviewer 1 Comments for the Author:
The requirement of Fz3 for the formation and polarization of Vangl2-Pk3 complex in the neural plate was not convincingly demonstrated in the manuscript. A number of issues need to be addressed as listed below.

Major points:
1. Consistent with previous observations from the authors, Figure 1 nicely shows anterior accumulation of Vang2 in CoMO-injected neural plate cells. Knockdown of Fz3 perturbs the anterior accumulation of Vangl2, however, this also disrupts Vangl2 membrane localization. Thus it is not clear whether the absence of anterior Vangl2 in Fz3 morphant cells is simply due to the absence of Vangl2 membrane localization. This point needs to be clarified. In addition, it may be also necessary to examine how Fz3 overexpression affects the anterior accumulation or membrane localization of Vangl2.

To distinguish the effects of Fz3 knockdown on Vangl2 anterior accumulation and membrane localization, we quantified fluorescence intensity of immunostained endogenous Vangl2 at lateral cell borders. We observed no significant difference between Fz3MO-injected and control uninjected cells (Figure S1A, B, page 4). We also found similar membrane localization of Vangl2 in Fz3-overexpressing superficial ectoderm cells (data not shown). These results suggest that the membrane localization of Vangl2 is not affected by Fz3.

2. What is the localization of Fz3 in neural plate cells? The Fz3 antibody seems to work in western blot, the authors may also try to see if it works in IF. Otherwise, the localization of exogenous Fz3 may be examined.

As the referee recommended, we examined Fz3 by immunostaining. Although endogenous Fz3 can be detected by immunoblotting, the signal was not visible by immunofluorescence, most likely due to low levels of expression. By contrast, exogenous Fz3 was readily detectable in neural plate cryosections with Fz3 antibodies. Notably, exogenous Fz3 was not planar polarized as might be expected for a PCP protein (Figure 1 for reviewers). We propose that additional factors are required to localize Fz3 at the posterior border of each cell. We decided not to include this preliminary result in the manuscript but will address this issue in a future study.

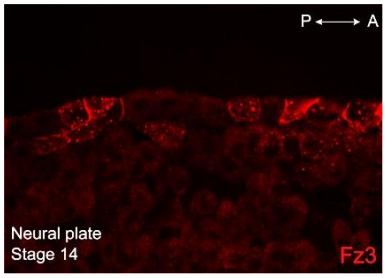

Figure 1 for reviewers. Subcellular localization of exogenous Fz3 in the *Xenopus* neural plate. (A-B) Two dorsal blastomeres of 16-cell embryos were injected with 25 pg of Fz3-FLAG mRNA. Embryos were cultured until stage 14, cryosectioned and immunostained with a mouse anti-Fz3 monoclonal antibody. The anteroposterior (A-P) axis is indicated.

3. Although biochemical analyses show that knockdown of Fz3 or expression of nonphosphorylatable Vangl2 enhances Vangl2-Pk3 complex formation, the influence of Fz3 on Vangl2-Pk3 localization in the neural plate is also unclear. It may be necessary to analyze how Vangl2-Pk3 complex is localized in neural plate cells following Fz3 overexpression and knockdown.

We analyzed early gastrula superficial ectoderm to avoid indirect effects of Fz3 on cell morphology at later stages. Overexpressed Fz3 strongly inhibited cortical localization of Pk3 (new Figure S3, page 7), consistent with our biochemical analysis (Figures 2A and 6B). Due to potential non-specific effects of Fz3MO on exogenous Vangl2 and Pk3 protein levels, we only studied Vangl2-Pk3 complexes upon overexpression of Fz3.

4. Page 8: “Vangl2 was compared with Vangl2-7A, which does not dissociate from the Pk3 protein in response to Fz3”. This is clearly shown by co-immunoprecipitation assay (Figure 6C). However, data from in vivo experiments are not fully consistent with the results from biochemical analyses. While Vangl2 and Pk3 showed exclusive complex formation and co-localization in the neural plate (Figure 7A,A’), co-expression of the nonphosphorylatable Vangl2-7A with Pk3 not only disrupted anterior accumulation but also caused less co-localization (Figure 7B,B’), suggesting reduced association.

The referee is correct, the discrepancy between our biochemical and colocalization data was due to the images in Figure 7 that did not fairly represent the observed outcome. To resolve this apparent contradiction, we included better images in the revision. The revised Figure 7 shows that the complexes of Vangl2-7A and Pk3 still form but lack proper polarity

5. In the model presented in Figure 7D, it is not easy to understand the way by which posteriorly localized Fz3 inhibits Vangl2-Pk3 complex formation and anterior localization. How does negatively regulatory feedback between Fz3 and Vangl2 lead to their segregation to opposite cell sides? This is not adequately discussed in the legend or in main text.

To address this comment, we modified Figure 7D and provide a more detailed description of the model in the Figure legend and the main text (pages 8 and 9). We explain that Fz3 promotes the phosphorylation of Vangl2 and the dissociation of the Vangl2-Pk3 complex in the proximity of the Fz3-enriched posterior domain. Therefore, Vangl2/Pk3 complexes are retained only at the anterior cortex.

Minor points:

1. Pages 5-6: “significant lower amounts of Vangl2 coprecipitated with Pk3 from Fz3-expressing explants”. Figure 3B needs quantification given that an important conclusion of this study is the inhibitory role of Fz3 on the association of Vangl2 and Pk3 in Xenopus embryos.

Prompted by the referee, we quantified the amounts of Vangl2 pulled down by Pk3 in embryos with or without Fz3. As shown in the revised Figure, Fz3 overexpression caused a 2.5-fold decrease in the binding of Vangl2 to Pk3.

2. The anterior localization of Vangl2 with respect to neural plate convergence and extension may be discussed further.

We updated the last paragraph of Discussion (pp 10, 11) as recommended.

Reviewer 2 Advance Summary and Potential Significance to Field:
The authors use proximity labeling and covalent crosslinking to show that the planar cell polarity proteins Vangl2 and Pk3 interact and that this interaction is disrupted by Fz3. Fz3 also disrupts the asymmetric distribution Vangl2 in Xenopus neural ectoderm. Their conclusions are supported by overexpression and morpholino depletion of Fz3. Fz3 overexpression also decreases the electrophoretic mobility of Vangl2, which they hypothesize (reasonably) is due to phosphorylation induced by Fz3; site directed mutagenesis of Vangl2 identifies residues that are necessary for phosphorylation of Vangl2 and disruption of the Vangl2/Pk3 complex. Overall, this work is interesting and moves the PCP field forward by defining a link between frizzled function and Vangl2/Prickle3 interaction in the developing embryo. There are some limitations to their interpretation that should be addressed by changes to the text but otherwise this work is a valuable contribution to the field.

Reviewer 2 Comments for the Author:
1. My main concern about their interpretation is the claim that T76 and T78 in Vangl2 are sites of Fz3-induced phosphorylation. While this is a reasonable hypothesis, they have not shown this. The T76/T78AA mutations may affect phosphorylation at other sites without being the primary site of Fz3-induced phosphorylation. Their own data show that mutation of serines 82 and 84 impairs phosphorylation at other sites (threonines, based on phospho-threonine immunoblotting (Fig. 5B)), clearly demonstrating that a mutation at one site can affect phosphorylation at other sites. They have shown that T76 and T78 are necessary, not that they are sites of phosphorylation, and certainly not that they are the primary sites of Fz3-induced phosphorylation. This could be addressed by changes to the text to remove this claim throughout the manuscript.

We propose that T76 and T78 are the sites of Fz-induced phosphorylation of Vangl2, based on Figure 5C showing that threonine phosphorylation was not induced in the T76T78>AA mutant, and given lack of other candidate threonine sites in the two N-terminal S/T clusters. Additionally, the removal of cluster II serine sites did not affect the ability of Fz3 to induce threonine phosphorylation. Following the suggestion from the reviewer, we modified the text throughout the manuscript (pp. 7 and 9) to soften our claim.

2. If “Fz3 inhibits Vangl2-Pk3 complex formation by triggering Vangl2 phosphorylation,” then why doesn’t Fz3 knockdown (by morpholino) reduce Vangl2 phosphorylation (Fig. 4B)? This can be addressed in the text and does not require new experiments.

Lack of significant changes of Vangl2 mobility in Fz3 morphants (Figure 4) may be due to limited assay sensitivity, as we now point out in revised Discussion (page 9, bottom). The gel shift assay might not allow to detect small changes in specific phosphorylation sites of Vangl2.

3. Their interpretation of the 7A and 5A mutations in cluster II is unclear. Perhaps this was a typographical error: “The association of Pk3 with Vangl2-7A was completely resistant to Fz3, whereas the complex containing Vangl2- 5A was partially sensitive (Fig. 6A, C). These experiments indicate that the T76T78 phosphorylation is necessary for the inhibitory effect of Fz3 on PCP complex formation.” If the 5A mutations have a partial effect, then don’t they mean that both T76/T78 and the serines in cluster II contribute? Or do they interpret the small effect of the cluster II mutations to mean their contribution is minimal and should be ignored? This should be clarified, as the implication as written now is that the cluster II mutations have no role.

Thank you for pointing out this inconsistency. We corrected the text on page 8 to acknowledge the “contributing role of cluster II serine residues for the inhibitory effect of Fz3 on PCP complex formation”.

4. As the Fz3 morpholino has a strong effect on Vangl2 localization, the authors should comment on the morphological phenotype of morpholino injected embryos. Furthermore, they note that nonphosphorylatable Vangl2 disrupts neural tube closure. If this is a real effect and not an artifact,

they should show these results. If they have concern that the effect is nonspecific, then this claim should be removed from the text.

As requested, we present the morphological phenotypes of Fz3 morphants (new Figure S2, page 4). We also added new Figure S4 (pp 7, 8) demonstrating neural tube defects in embryos expressing the T76T78>AA Vangl2 mutant.

Second decision letter

MS ID#: JOCES/2021/258864

MS TITLE: Frizzled3 inhibits Vangl2-Prickle3 association to establish planar cell polarity in the vertebrate neural plate

AUTHORS: Ilya Chuykin, Kyeongmi Kim, and Sergei Sokol
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 gave favourable reports but raised some critical points 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, depending on further comments from reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. 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

This manuscript reports a role of Fz3 for the establishment of PCP in the neural plate of Xenopus embryos.
The authors showed that Fz3 regulates the interaction between Vangl2 and Pk3 through Vangl2 phosphorylation on T76 and T78. Overexpression of Fz3 suppresses, whereas knockdown of Fz3 enhances Vangl2 and Pk3 association. A nonphosphorylatable form of Vangl2 fails to polarize with Pk3 in neural plate cells. Based on these observations, the authors postulate that Fz3 regulates the formation and anterior localization of Vangl2-Pk3 complex by inducing the phosphorylation of T76 and T78 in Vangl2. Results from this study raise the possibility that Fz3-mediated Vangl2 phosphorylation at specific sites may participate in the establishment of epithelial polarity. They

could help to understand the regulation of neural plate PCP and would be of potential interest to the fields of Wnt signaling as well as cell and developmental biology.

Comments for the author

The authors adequately revised the manuscript, which is now improved and appropriate for publication

Reviewer 2

Advance summary and potential significance to field

No change from previous comments.

Comments for the author

The authors have not adequately addressed my main concern (point #1 on previous review). This is unfortunate, as the comment was reasonable and only required simple edits to the manuscript. They have NOT shown that Fz3 induces phosphorylation of T76 and T78. There is nothing wrong with hypothesizing or proposing the sites, as they do in a few places, but it should not be stated as fact, as they do prominently in the abstract, subheadings, figure legend title and other instances:
Abstract: “In addition, Fz3 induced Vangl2 phosphorylation on T76 and T78…”
P4: “This function of Fz3 depends on the phosphorylation of the conserved T76 and T78 residues of Vangl2…”
P7: “These observations indicate that T76 and T78, unlike S82 and S84, are phosphorylated in response to Fz3 signaling.”
P7: “Vangl2 T76/T78 phosphorylation is required for the inhibitory activity of Fz3…”
P8: “uncovering roles of T76 and T78 phosphorylation in this process...”
P10: “the analysis of specific phosphorylation sites, such as T76 and T78.”
P10: “the phosphorylation of T76T78 is inducible…”
P21: “Figure 5. Fz3 induces the phosphorylation of T76 and T78 in Vangl2.”
P22:” (C) Fz3 induces phosphothreonine phosphorylation of the T76T78 sites in Vangl2.”
This only requires simple editing to fix this.

Second revision

Author response to reviewers' comments

Response to Reviewer 2

Reviewer 2 Advance Summary and Potential Significance to Field:
No change from previous comments.

Reviewer 2 Comments for the Author:
The authors have not adequately addressed my main concern (point #1 on previous review). This is unfortunate, as the comment was reasonable and only required simple edits to the manuscript. They have NOT shown that Fz3 induces phosphorylation of T76 and T78. There is nothing wrong with hypothesizing or proposing the sites, as they do in a few places, but it should not be stated as fact, as they do prominently in the abstract, subheadings, figure legend title, and other instances:

Abstract: “In addition, Fz3 induced Vangl2 phosphorylation on T76 and T78…”
P4: “This function of Fz3 depends on the phosphorylation of the conserved T76 and T78 residues of Vangl2…”
P7: “These observations indicate that T76 and T78, unlike S82 and S84, are phosphorylated in response to Fz3 signaling.”
P7: “Vangl2 T76/T78 phosphorylation is required for the inhibitory activity of Fz3…”

P8: “uncovering roles of T76 and T78 phosphorylation in this process...”
P10: “the analysis of specific phosphorylation sites, such as T76 and T78.”
P10: “the phosphorylation of T76T78 is inducible…”
P21: “Figure 5. Fz3 induces the phosphorylation of T76 and T78 in Vangl2.”
P22:” (C) Fz3 induces phosphothreonine phosphorylation of the T76T78 sites in Vangl2.”

This only requires simple editing to fix this.

We acknowledge that initially we did not fully understand the point made by the referee. However, since the comment is completely valid, we changed text in the abstract, subheadings, figure legend title and the main text, as requested by the reviewer. The claim that Fz3 directly induces T76 and T78 Vangl2 phosphorylation has been removed from the manuscript. All changes in the amended manuscript file have been highlighted.

Third decision letter

MS ID#: JOCES/2021/258864

MS TITLE: Frizzled3 inhibits Vangl2-Prickle3 association to establish planar cell polarity in the vertebrate neural plate

AUTHORS: Ilya Chuykin, Keiji Itoh, Kyeongmi Kim, and Sergei Sokol
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.

Reviewer 2

Advance summary and potential significance to field

See previous review. No further comments.

Comments for the author

The authors have addressed my concerns completely. No further comments. It is interesting work.