# The Glycoprotein GP130 governs the Surface Presentation of the G-Protein Coupled Receptor APLNR

Kilian Trillet, Kathryn Jacobs, Gwennan Andre-Gregoire, An Thys, Clément Maghe, Jonathan Cruard, Stephane Minvielle, Sara Gonzalez-Diest, Guillaume Montagnac, Nicolas Bidere, and Julie Gavard

*Corresponding Author(s): Julie Gavard, INSERM, CNRS, Université de Nantes*

## Review Timeline:

| | |
|---|---|
| Submission Date: | 2020-04-16 |
| Editorial Decision: | 2020-06-17 |
| Revision Received: | 2020-12-29 |
| Editorial Decision: | 2021-01-25 |
| Revision Received: | 2021-04-25 |
| Editorial Decision: | 2021-05-14 |
| Revision Received: | 2021-05-18 |

*Monitoring Editor: Pier Paolo Di Fiore*

*Scientific Editor: Tim Spencer*

## 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.)

**DOI: https://doi.org/10.1083/jcb.202004114**

June 17, 2020

Re: JCB manuscript #202004114

Dr. Julie Gavard
INSERM, CNRS, Université de Nantes
8 quai Moncousu
Nantes 44000
France

Dear Dr. Gavard,

Thank you for submitting your manuscript entitled "The Glycoprotein GP130 governs the Surface Presentation of the G-Protein Coupled Receptor APLNR". The manuscript has been evaluated by expert reviewers, whose reports are appended below. Unfortunately, after an assessment of the reviewer feedback, our editorial decision is against publication in JCB.

You will see that although two of the reviewers have voiced some enthusiasm for the premise of the study, they each raise a number of substantive concerns which preclude publication at this time. In particular, the reviewers feel that the study is too preliminary at this stage and significantly more data is needed to sufficiently support the conclusions.

Given the scale of the revisions that will be needed to make the paper suitable for publication in JCB as well as the complications that have arisen due to the efforts of many universities to slow the spread of COVID-19, it may be best to pursue publication at another journal - particularly if you wish to expedite publication of the current data

However, we would be open to receiving an appeal of this decision at a later date which includes a significantly revised and extended manuscript that fully addresses each of the reviewers' concerns. Please note, though, that such a resubmission would be subject to evaluation for priority and novelty, as well as a second round of peer review. In particular, we feel that nearly all of the reviewer concerns will need to be addressed in a revised manuscript.

If you choose to file an appeal, we will strive to use the same reviewers. However, please note that we may need to recruit a new reviewer to assess the revision if the original reviewers are unable to re-review or if further expert opinion on the work is needed.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. You can contact the journal office with any questions, cellbio@rockefeller.edu.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

Pier Paolo Di Fiore, MD, PhD
Editor

The Journal of Cell Biology

Tim Spencer, PhD
Executive Editor
Journal of Cell Biology

---------------------------------------------------------------------------

Reviewer #1 (Comments to the Authors (Required)):

Trillet et al. "The glycoprotein GP130 governs the surface presentation of the G-protein coupled receptor APLNR"

In this manuscript, experiments testing the ideas that GP130 binds to APLNR on the plasma membrane of glioblastoma stem cells with a positive feedback loop of an increase in ELMOD1 expression leading to an recycling of GP130 and APLNR to the plasma membrane. Evidence is provided for interaction of GP130 and APLNR in PM microdomains in glioblastoma stem cells. Stem cell expansion requires GP130 in patient derived GSCs. GP130 increases the level of APLNR at the PM. To define the mechanism, they examined changes in the transcriptome on GP130 deletion. One gene they found was ELMOD1, a GAP for Arf family GTPases. ELMOD1 was found to affect trafficking of APLNR to the cell surface and, based on TCGA data, ELMOD1 and GP130 expression levels affected patient prognosis. The results and proposed mechanism are interesting, but the work is preliminary in two areas that should be addressed before publication,

First, although many approaches are used to test the idea of direct interaction between APLNR (and other receptors) and GP130, the data are not particularly compelling and details, such as number of replicate experiments and summary of several experiments are not included. E.g. in Figure 1A, is it possible to provide quantification, a pearson's coefficient or similar and a fraction of colocalized protein; in Figure 1B, the results with IL6R are not clear (bands not distinct) and for all the proteins tested, there is no sense of inter experiment variation - can you supply some summary data, maybe scans? ; in figure 1C, some summary data - number of experiment, quantification of signal; in figure 1D, the bands don't match for APLNR. There is also no indication of reproducibility; figure 1G, how many cells analyzed? Any quantification?

Second, the connection with Arf6 and ELMOD1 is poorly developed. Importantly, the biggest effect of NAV2729 has been found to be on Arf1, not Arf6 and it is a nonspecific inhibitor of Arf GEFs. It cannot be used by itself to assert an Arf6 mechanism. Similarly, although ELMOD1 was reported to have a preference for Arf6 among GTPases tested, it can also use Arl1 and many other GTPases have not been tested. The ideas of ELMOD1 mediating effects of GP130 are not sufficiently developed.

Third, ELMOD1 overexpression is associated with poorer prognosis (note state the opposite in the text, a minor issue) but there is no analysis of genes that might be coamplified, potentially explaining the observation.

Reviewer #2 (Comments to the Authors (Required)):

Trillet et al JCB 2020

This manuscript describes an important role for the glycoprotein gp130 in the surface expression and consequent signaling activity of the G-protein coupled receptor APLNR. Previous work from the authors' lab has shown that the ligand for APLNR, the vasoactive peptide apelin, is critical for the maintenance and growth of glioblastoma stem-like cells (GSCs), suggesting that the apelin/APLNR signaling axis may be a druggable target for intervention in glioblastoma.

Here the authors show that APLNR associates constitutively with gp130, an obligate component of several other cytokine receptors (including IL-6R) that is important for coupling of these receptors to downstream JAK/STAT signaling. Knockdown or knockout of GP130 substantially reduces the surface level of APLNR, and correspondingly attenuates apelin-induced signaling. RNAseq analysis of GP130-deficient cells suggested that expression of ELMOD1, a GAP for small GTPases of the ARF/Arl family, is significantly reduced compared to control cells. This is consistent with the presence of multiple STAT3 binding sites proximal to the ELMOD1 gene (but also present near the ELMOD2 and ELMOD3 genes - more on this below).

The coupling of APLNR with gp130 and its importance in controlling APLNR surface expression/signaling is convincingly demonstrated. However, the authors go on to suggest that ELMOD1 controls trafficking of the APLNR/gp130 complex by reducing the activity of the ARF family member ARF6, thereby inhibiting APLNR/gp130 endocytosis. This aspect of the study is much less convincing. While it is clear that reducing the level of ELMOD1 significantly impacts the surface expression of APLNR/gp130, it is not at all clear how this is achieved mechanistically:
1. The authors never directly measure the rate of APLNR/gp130 endocytosis. Increased surface APLNR/gp130 could also be due to enhanced recycling, which would be more consistent with known functions of ARF6. It is also possible that gp130 is required for efficient exit of APLNR from the ER, but this possibility is not explored. Does newly synthesized APLNR associate with gp130?
2. While the authors describe ELMOD1 as a GAP for ARF6, it also has activity toward other ARF family members, including other ARFs and ARF-like proteins (Arls), which are not analyzed here. In fact, most of the assumptions about substrate specificity of the ELMODs are based on a single in vitro analysis by Kahn and colleagues, and have not been validated in intact cells. In agreement with this, the level of active ARF6 is barely changed in gp130-deficient cells (Fig. 5D). Moreover the graph does not appear to correlate with the immunoblot shown. In order to claim that ELMOD1 is acting through ARF6 the authors must deplete cells of ARF6 directly and measure endocytosis/recycling of APLNR/gp130 as noted above.
3. Similarly, the drug NAV2729 inhibits the activity of several ARF GEFs that themselves act on more than one ARF, again emphasizing the need for direct targeting of ARF6 to support the authors' claims.
4. The authors state that all 3 ELMOD genes contain adjacent STAT3 binding sites, implying that all 3 ELMOD isoforms are similarly regulated by STAT3. It is therefore unclear why the authors chose to focus on ELMOD1. In fact, ELMOD2 has significantly higher specific activity toward ARF6 in vitro than does ELMOD1. Do the other ELMODs similarly affect APLNR/gp130 trafficking?
5. Referring to Fig. 5I, the authors state that high levels of ELMOD1 expression correlate with better prognosis in GBM patients, yet the figure suggests the opposite. Is the figure mislabeled or is their statement incorrect?

Minor point - ARNO is one of many GEFs for ARF6 including other cytohesins, IQSecs and EFA6 isoforms. Fig. 5A shows ARNO as the GEF that is inhibited by NAV 2729, but no evidence is presented in support of this claim.

Julie Gavard
SOAP, Signaling in Oncogenesis, Angiogenesis and Permeability
CRCINA, INSERM UMR1232, CNRS ERL6001
Universite de Nantes
8 quai Moncousu BP70721
48007 Nantes Cedex 1
France
Ph: + 33 22 808 0327
Email: julie.gavard@inserm.fr

Dear Editor,

Please let me first thank you and the Reviewers for your hard work and positive comments on our manuscript. We were glad to see that all acknowledge the interest of the findings and the quality of the experimental data.

At your suggestion, we now submit an appeal of the decision and include an extensive revised version of our work, together with a point-by-point rebuttal. We have addressed the Reviewers' concerns with additional experiments and controls, included in this revised version that now contains 6 main figures and 7 supplementary figures. Notably, we have performed additional work and now provide a more comprehensive view on the mechanisms involved in modulating APLNR surface presentation in glioblastoma patient cells. By combining multiple means to track APLNR internalization with flow cytometry (Fig 3I, 5C, 6E) and confocal microscopy (Fig 3G), we conclusively show that GP130 expression governs APLNR endocytosis via the GTPase activating protein ELMOD1, (Fig 5). In this context, we have also evaluated in more details the molecular contribution of ARF6 to the GP130 phenotype with RNAi-mediated silencing of these ARFs in patient cells (Fig 6C-F). In addition, we find that ELMOD1, but not sibling ELMOD2 and ELMOD3, is the main mediator of this phenotype (Fig 4D, 5D). In-depth data mining also unmasked a specific signature related to transport, nucleotide, and synapse, associated with *ELMOD1* gene expression in cancer patients (Fig 4H). We also clarified number of replicates and cells, and quantified most of our data in order to better appreciate the robustness of the observed phenotype (Fig 1A-D, 1G, 2A-B, 2D, 2I, 3A-D, 3F, 3I, 5B-D, 6A-B, 6D-E).

We thank you and the Referees for your helpful comments, which have further clarified the manuscript and strengthened our conclusions, and we trust that it is now suitable for publication in *The Journal of Cell Biology*.

Below is our point-by-point discussion in which we reiterate the Reviewers' comments and provide a point-by-point rebuttal.

Sincerely,
Julie Gavard

**Reviewer #1**

We thank the Reviewer for his/her useful comments on our manuscript and for his/her statement that "The results and proposed mechanism are interesting". We have added essential experiments and missing controls to provide more insights at the mechanistic level, especially the interplay between ARFs and APLNR internalization.

1. First, although many approaches are used to test the idea of direct interaction between APLNR (and other receptors) and GP130, the data are not particularly compelling and details, such as number of replicate experiments and summary of several experiments are not included.
E.g. in Figure 1A, is it possible to provide quantification, a pearson's coefficient or similar and a fraction of colocalized protein; in Figure 1B, the results with IL6R are not clear (bands not distinct) and for all the proteins tested, there is no sense of inter experiment variation - can you supply some summary data, maybe scans? ; in figure 1C, some summary data - number of experiment, quantification of signal; in figure 1D, the bands don't match for APLNR. There is also no indication of reproducibility; figure 1G, how many cells analyzed? Any quantification?
We thank the Reviewer for this remark and would like to take this opportunity to apologize for not providing enough information in our initial manuscript. Figure legends now clearly state for each panel the number of replicates and the number of cells analyzed, when required. In addition, quantification analysis was performed and is now included in most panels. At the Reviewer's request, Pearson's coefficient is also provided for Figure 1A.
After revisiting our data, we agree that the experiments initially presented Figures 1B and 1D may be difficult to interpret. Repeat experiments together with a densitometry analysis on independent experiments are now included. At the Reviewer's suggestion, we also provide quantification of number of dots per cell in Figure 1C. Likewise, Figure 1G is now supplemented with intensity histogram, representative of >10 cells.

2. Second, the connection with Arf6 and ELMOD1 is poorly developed. Importantly, the biggest effect of NAV2729 has been found to be on Arf1, not Arf6 and it is a nonspecific inhibitor of Arf GEFs. It cannot be used by itself to assert an Arf6 mechanism. Similarly, although ELMOD1 was reported to have a preference for Arf6 among GTPases tested, it can also use Arl1 and many other GTPases have not been tested. The ideas of ELMOD1 mediating effects of GP130 are not sufficiently developed.
These are very insightful concerns, also shared by Reviewer#2 (please, see his/her point#2), that we tried to experimentally address. As inferred by the Reviewers, the loss of ELMOD1 expression, observed in GP130 knockout cells, correlates with an increase ARF6 GTP-loading (new Figure 6A). Moreover, *ARF6* silencing provokes a decrease in APLNR surface presentation and an

increase in anti-APLNR antibodies uptake, consistent with a decrease endocytosis rate (new Figures 6C-6E). ARF6 knockdown was thus sufficient to recapitulate the effects of ELMOD1 silencing, including on APLN-mediated tumorsphere formation (new Figure 6F). Moreover, the expression of ARF6-Q67L mutant recapitulates the phenotype observed in *GP130* or *ELMOD1* knockdowns (new Figure 6B), favoring thus the involvement of this GTPase in controlling APLNR localization. Although the implication of other GTPases, including ARF1 Arl1, cannot be ruled out, our data conclusively demonstrate a crucial role played by ARFs and ELMOD1. Nevertheless, this possibility is now discussed in the revised manuscript.

3. Third, ELMOD1 overexpression is associated with poorer prognosis (note state the opposite in the text, a minor issue) but there is no analysis of genes that might be coamplified, potentially explaining the observation.

We thank the Reviewer for catching this mistake. This is now corrected. In addition, we have explored TCGA for co-regulated genes based on ELMOD1 expression. This datamining unveiled that ELMOD1 is associated with gene signature linked to 635 up-regulated genes and 257 down-regulated genes. At the functional scale, ELMOD1 clusters with functions related to transport, nucleotide, synapse, cell cycle, and ion flux (new Figure 4H). This is thus mainly aligned with its reported functions as a GTPase-activating protein (GAP).

-------------

**Reviewer #2**

We thank this Reviewer for his/her constructive comments on our manuscript. At his/her suggestion, we have performed substantial additional experiments to clarify the mechanisms by which ELMOD1 modulates APLNR/GP130 surface presentation.

1. The authors never directly measure the rate of APLNR/gp130 endocytosis. Increased surface APLNR/gp130 could also be due to enhanced recycling, which would be more consistent with known functions of ARF6. It is also possible that gp130 is required for efficient exit of APLNR from the ER, but this possibility is not explored. Does newly synthesized APLNR associate with gp130?

This is an important point and we have now implemented several strategies to define the effect of GP130 knockout on APLNR surface expression. First, our analysis of endocytosis rate by flow cytometry shows an exacerbated internalization of APLNR in GP130 knockout cells, as evaluated with antibody uptake (new Figure 3H-I). Confocal microscopy analysis further reveals that internalized anti-APLNR antibodies coalesce within Rab5-positive vesicles, but not with Rab7 ones, nor LAMP2 (new Figure 3H and data not shown). Similar effects on APLNR internalization were seen in both *ELMOD1* and *ARF6* knocked down cells, favoring a model in which endocytosis is exacerbated (new Figures 5C and 6E). In addition, the expression of ARF6 Q67L mutant, which

accumulates in intracellular vesicles, reduces APLNR surface presentation, similarly to *ELMOD1* silencing (new Figure 6B). Of note, the expression of the ARF6 T27N mutant (in recycling endosomes) slightly enhances APLNR at the plasma membrane. This suggests that ARF6 contributes to ligand-independent endocytosis of APLNR, although the GTPase may bounce back on APLNR recycling.

We also analyzed the glycosylation status of both APLNR and GP130 in an ectopic system and found that the fraction of EndoH-sensitive APLNR is not noticeably modified by GP130 expression, suggesting that exit from ER is not linked to GP130 expression (Figure S5D). Although we agree with the Reviewer that assessing whether GP130 and APLNR associate at early stage of synthesis would be of clear interest, we feel that it will fall beyond the scope of the current manuscript. Nevertheless, this interesting option is now discussed in the revised manuscript. Of note, a portion of GP130 staining was found stuck intracellularly, without APLNR (data not shown), suggesting additional fate for GP130 independent of APLNR trafficking.

2. While the authors describe ELMOD1 as a GAP for ARF6, it also has activity toward other ARF family members, including other ARFs and ARF-like proteins (Arls), which are not analyzed here. In fact, most of the assumptions about substrate specificity of the ELMODs are based on a single in vitro analysis by Kahn and colleagues, and have not been validated in intact cells. In agreement with this, the level of active ARF6 is barely changed in gp130-deficient cells (Fig. 5D). Moreover the graph does not appear to correlate with the immunoblot shown. In order to claim that ELMOD1 is acting through ARF6 the authors must deplete cells of ARF6 directly and measure endocytosis/recycling of APLNR/gp130 as noted above.

Most of these insightful suggestions were shared with Reviewer#1, and several aspects are therefore discussed in our response to Reviewer#1, point#2. In addition, the data presented in the new Figure 6A were replaced with repeat experiments, together with densitometric analysis, suggesting that ELMOD1 loss of expression (as observed in GP130 knockout cells) correlates with an increase in ARF6 GTP loading. At the reviewer's suggestion, we measured APLNR surface presentation and endocytosis in ARF6 knockdown cells, and found that it phenocopied ELMOD1 silencing (new Figure 6C-E). At the functional level, ARF6 silencing hinders APLN-mediated tumorsphere formation (new Figure 6F).

3. Similarly, the drug NAV2729 inhibits the activity of several ARF GEFs that themselves act on more than one ARF, again emphasizing the need for direct targeting of ARF6 to support the authors' claims.

This is a valid point, also raised by Reviewer#1 (please, see our response to his/her point#2).

4. The authors state that all 3 ELMOD genes contain adjacent STAT3 binding sites, implying that all 3 ELMOD isoforms are similarly regulated by STAT3. It is therefore unclear why the authors

chose to focus on ELMOD1. In fact, ELMOD2 has significantly higher specific activity toward ARF6 in vitro than does ELMOD1. Do the other ELMODs similarly affect APLNR/gp130 trafficking?

This is an interesting remark, and several lines of experiments were carried out to evaluate the interplay between ELMODs and GP130. First, our analysis of ELMODs mRNA by qPCR revealed that only ELMOD1 appears under the direct control of GP130 in patient cells (new Figure 4D). Further militating against a role for ELMOD2 and ELMOD3, their silencing with siRNA had no overt effect on APLNR surface expression (new Figure 6D). Hence, our data suggest a specific role of ELMOD1 in orchestrating APLNR trafficking.

5. Referring to Fig. 5I, the authors state that high levels of ELMOD1 expression correlate with better prognosis in GBM patients, yet the figure suggests the opposite. Is the figure mislabeled or is their statement incorrect?

We thank the Reviewer for catching this mistake. This is now corrected.

Minor point - ARNO is one of many GEFs for ARF6 including other cytohesins, IQSecs and EFA6 isoforms. Fig. 5A shows ARNO as the GEF that is inhibited by NAV 2729, but no evidence is presented in support of this claim.

This reference has been removed and the possible involvement of GEFs such as ARNO, cytohesins, IQSecs and EFA6 are now discussed.

January 21, 2021

Re: JCB manuscript #202004114R-A

Dr. Julie Gavard
INSERM, CNRS, Université de Nantes
8 quai Moncousu
Nantes 44000
France

Dear Dr. Gavard,

Thank you for submitting your revised manuscript entitled "The Glycoprotein GP130 governs the Surface Presentation of the G-Protein Coupled Receptor APLNR". The manuscript has been seen by the original reviewers whose full comments are appended below. While the reviewers continue to be overall positive about the work and believe that the manuscript has made substantial progress, some important issues remain and, thus, we are unable to publish the paper in its current form.

You will see that both reviewers feel that the data are inconsistent with your proposed model which suggest that ELMOD1 negatively regulates ARF6 at steady state and this, in turn, modulates surface expression of APLNR. As noted by reviewer #2, the data instead suggests that "ARF6 is not the key GTPase that controls APLNR endocytosis" and that it "more likely...controls APLNR recycling rather than endocytosis." Both we and the reviewers feel that this issue needs to be conclusively addressed via new experiments. Thus, if you wish to publish this work quickly, it may be in your best interests to submit the manuscript elsewhere.

In addition, as you may know, our general policy is that papers are considered through only one revision cycle. However, given that the reviewers have voiced significant enthusiasm for the underlying premise of the study, we are willing to make a rare exception to this rule and allow you to submit one final revision, provided that you are able to address this and the other reviewer concerns in full with new data. However, please note that the paper will be sent back to the reviewers for final assessment and we will need to see that the reviewers are fully satisfied before we can commit to publication. If they do not find the revisions sufficient, we will be forced to reject the paper and will not be able to consider any further iterations of the work. Given this scenario, we strongly recommend that you send us a revision plan which illustrates how you plan to address the remaining reviewer comments prior to beginning any new experiments.

Also note that we are happy to transfer the reviewer comments to any other journal. Thus, if you would instead prefer to take the manuscript elsewhere and transfer the comments, just let us know.

Regardless of what you decide, thank you for this interesting contribution to Journal of Cell Biology. You can contact me or the scientific editor listed below at the journal office with any questions, cellbio@rockefeller.edu.

Sincerely,

Pier Paolo Di Fiore, MD, PhD
Senior Editor
The Journal of Cell Biology

Tim Spencer, PhD
Executive Editor
Journal of Cell Biology

------------------------------------------------------------------------

Reviewer #1 (Comments to the Authors (Required)):

Trillet et al

Many aspects of the manuscript have been improved; however, the section with the Arf pathway is preliminary with conflicting results. Importantly,

1. ELMOD1 causes the hydrolysis of GTP bound to Arf. Thus, loss of ELMOD1 would increase the levels of Arf-GTP, the putative active form. Arf knockdown and Arf exchange factor inhibitor would decrease the levels of Arf-GTP. Therefore, the predicition is these two treatments would have the opposite result as loss of ELMOD1, but the data indicate all three treatments have the same effect.
2. NAV2729 works most effectively as an inhibitor of BRAG2 and Arf1 activation, not Arf6. See Benabdi et al (2017) Biochemistry 56, 5125.
3. Dominant negative and constitutively active forms of Arf often have significant indirect effects.

Reviewer #2 (Comments to the Authors (Required)):

This revised manuscript is improved over the original through the addition of significant new data. Specifically, the authors show that loss of GP130 leads to redistribution of the apelin receptor APLNR into endosomal compartments from the plasma membrane, presumably inhibiting its access to ligand and attenuating the apelin-induced signaling response. In response to reviewer concerns, the authors now show that this redistribution is due to an increased rate of APLNR endocytosis. In attempting to determine the mechanism through which this occurs, they show that loss of GP130 leads to reduced expression of the ARF/ARL GAP ELMOD1, and consequent increased activity of the ARF family member ARF6. The authors propose a model in which GP130, acting through STAT3, transcriptionally enhances expression of ELMOD1, leading to reduced ARF6 activity and inhibition of APLNR endocytosis. The increased surface availability of APLNR leads to enhanced growth of glioblastoma tumor spheres, and high ELMOD1 expression correlates with reduced survival, presumably due to increased tumor growth.

While this model might make sense if the data supported it, there is a significant conceptual problem with the data provided. ELMOD1 is a GAP for ARF6 (as well as other ARFs/ARLs, see below), and the authors show convincingly that loss of ELMOD1 leads to increased ARF6 activity (new Fig. 6A). However, they also show that knockdown of ARF6 phenocopies loss of ELMOD1 or GP130 (reduced surface expression and increased endocytosis of APLNR). Thus loss of ARF6 has the same effect as increased activity of ARF6, which doesn't make mechanistic sense.

As noted above, ELMOD1 acts on multiple ARFs and ARF-related proteins (ARLs), and it therefore

seems likely that ARF6 is not the important target in this context. While both reviewers raised the issue of ELMOD1 substrate promiscuity in the first round of reviews, the authors have continued to focus solely on ARF6. Is activation of other ARFs similarly enhanced in the absence of ELMOD1? If so, does their depletion restore APLNR surface expression in the absence of ELMOD1?

May 14, 2021

RE: JCB Manuscript #202004114RR

Dr. Julie Gavard
INSERM, CNRS, Université de Nantes
8 quai Moncousu
Nantes 44000
France

Dear Julie:

Thank you for submitting your revised manuscript entitled "The Glycoprotein GP130 governs the Surface Presentation of the G-Protein Coupled Receptor APLNR". We have now had a chance to review the changes and we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Articles and Tools is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends. You are below the limit at this time but please bear it in mind when revising.

2) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

3) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, since you used parametric tests in your study (e.g. t-tests, ANOVA, etc.), you should have first determined whether the data was normally distributed before selecting that test. In the stats section of the methods, please indicate how you tested for normality. If you did not test for normality, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

4) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

5) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies.

6) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

7) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

8) Supplemental materials: There are normally strict limits on the allowable amount of supplemental data. Articles may usually have up to 5 supplemental figures. At the moment, you currently have 8 such figures. While we will be able to give you a bit more space in this case, we ask that you remove supplementary figure 1 and include the APLNR genomic sequence as simple text in the materials and methods section.
Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

9) eTOC summary: A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

10) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

11) A separate author contribution section is required following the Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames. We encourage use of the CRediT nomenclature (https://casrai.org/credit/).

12) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and MP4 video files: See our detailed guidelines for preparing your production-ready images, https://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

**It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.**

**The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.**

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7-14 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Pier Paolo Di Fiore, MD, PhD
Senior Editor
Journal of Cell Biology

Tim Spencer, PhD
Executive Editor
Journal of Cell Biology

--------------------------------------------------------------------------------