Protein tyrosine phosphatase 1B targets focal adhesion kinase and paxillin in cell-matrix adhesions

Ana E. González Wusener, Ángela González, María E. Perez Collado, Melina R. Maza, Ignacio J. General and Carlos O. Arregui
DOI: 10.1242/jcs.258769

Editor: Arnoud Sonnenberg

Review timeline
Original submission: 12 April 2021
Editorial decision: 20 May 2021
First revision received: 17 August 2021
Editorial decision: 9 September 2021
Second revision received: 13 September 2021
Accepted: 14 September 2021

Original submission

First decision letter

MS ID#: JOCES/2021/258769

MS TITLE: PROTEIN TYROSINE PHOSPHATASE 1B, PTP1B, TARGETS FAK AND PAXILLIN IN CELL-MATRIX ADHESIONS

AUTHORS: Ana E González Wusener, Ángela González, María E. Perez Collado, Melina R. Maza, Ignacio J General, and Carlos O. Arregui
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

PTP-1B is an ER associated tyrosine phosphatase with a demonstrated role in regulation of cell adhesion/migration. However, there is limited information regarding the identity of direct focal adhesion-associated PTP-1B substrates. Using a bimolecular fluorescence complementation (BiFC) approach, in combination with a substrate trapping PTP-1B DA mutant, the authors take a candidate approach to identify paxillin PY31/118 and FAK PY925 as probable targets. Electrostatic computer modeling predicts that PY925 dephosphorylation would facilitate paxillin-FAK binding, consistent with previous studies and this was supported by analysis of the interaction between pxn and FAK in WT vs PTP-1B KO cells. However, the levels of PY925 FAK were not significantly impacted in the KO cells nor was the previously reported downstream activation of Erk. Based on these studies, the authors propose a complex bimodal regulation of focal adhesion dynamics by PTP-1B, that would have benefited from confirmation by additional experimentation. In general, the BiFC analysis is well-executed and convincing. However, the outcome of PTP-1B regulation of pxn and FAK and their interaction remains largely speculative.

Comments for the author

Major points to be addressed

1. The BiFC analysis would benefit from the analysis of another tyrosine phosphorylated adhesion protein that is NOT a target for PTP-1B and thus would presumably not bind the CA mutant.

2. The analysis is largely unidimensional. Biochemical co-IP expts should be performed to confirm the specificity of the substrate trapping BiFC analysis.

3. PY blotting of the tagged pxn and FAK WT and mutant constructs should be performed from WT and KO PTP-1B cells. This would help further validate the proposed specificity of the FAK PY925 data, which is currently rather weak.

4. Can the authors explain why the BIFC labeling of focal adhesions is more robust and more focal adhesion-like (as opposed to punctate) with the FAK WT, Y397F, 3F mutants (fig 5C-H)?

5. The modeling of electrostatic effects on FAK conformation doesn't really add anything to the dataset and largely confirms already published experimental data.

6. The Discussion is overly detailed and speculative, and largely unsupported by new experimental data (eg. regulation of downstream signaling from Y925). It could be shortened considerably, and/or supported by additional experimentation.

Minor points

1. As noted in the discussion, PTP-PEST is another major phosphatase that regulates focal adhesions and it, like FAK also binds pxn (Shen JBC 1998, Cote et al JBC 1999,) and regulates pxn and Rac1 signaling (Jamieson JCS 2005). Discussion of the respective roles for PTP-PEST vs PTP-1B could be expanded to address their respective roles.

2. Is there any pxn in focal adhesions in the FAK KO cells? Could this explain the lack of a BiFC signal (Fig 3 K,L)? There also seems to be more a-GFP spots in this image than suggested by the quantitation (Fig 3 M).

3. Does the positioning of the BiFC constructs on opposite ends of the relevant molecules impact outcomes?

Reviewer 2

Advance summary and potential significance to field

The regulation of tyrosine phosphorylation of proteins in cell adhesions is critical for multiple biological processes. The kinases responsible for phosphorylation have been identified, but discovery of the phosphatases catalyzing dephosphorylation lags behind. The problem is further complicated by the presence of multiple sites of phosphorylation on these substrates.
This paper provides evidence that PTP1B may be responsible for dephosphorylation of select phosphotyrosines on FAK and paxillin in vivo by demonstrating a specific active site mediated interaction by BiFC. This provides novel mechanistic insight into the control of biological functions by PTP1B.

Comments for the author

The paper should be revised prior to publication.
1) expression levels of the constructs need to be validated. This is very convincingly done for paxillin in supplemental figure 1. This should also be shown for the paxillin mutants, wild type FAK and its mutants and the PTP1B constructs. The authors appear to have the data and this should be shown in additional supplemental figures. This is important to rule out effects due to overexpession of exogenous constructs.

2) the BiFC signal is not uniformly distributed in the adhesions. How does this compare to paxillin distribution and phosphorylated paxillin distribution? Have the authors looked at this or is it documented in the literature. Related, the BiFC signal between PTP1B and FAK appears different in distribution in adhesions that the BiFA signal between PTP1B and paxillin. Are there really differences?

3) BiFC in figures 2 and 4 is quite sharp and prominent in adhesions. In figure 3 and 5 there is more diffuse background staining. Why?

4) Supplemental figure 3 needs a control of cells in suspension, or other negative control, to validate adhesion dependent phosphorylation of Erk in their assay 5) The PTP1B KO cells show elevated interactions between FAK and paxillin that the authors ascribe to altered phosphorylation of Y925 on FAK and a change in the FAT domain conformation. This is not consistent with the modest, insignificant increase in phosphorylation of this site determined by blotting. This would also require high stoichiometry of phosphorylation.
Without this additional data, this conclusion is not strongly supported by the evidence. The observation is intriguing, but the authors need to consider and discuss other potential mechanisms as well, and these could be indirect.

Other minor (but important) edits:
1) the Flint reference and the description of pervanadate specifically blocking substrate trapping needs to appear in the results section when the experiment is first described.
2) what does the anti-GFP antibody recognize? YN, YC or both?
3) the authors should use the word "doubled" rather than "duplicated" to describe the level of expression of the exogenous constructs.

Reviewer 3

Advance summary and potential significance to field

Comments to manuscript by Gonzalez Wusener and coworkers:

The understanding of cell adhesion and migration, will involve the careful dissection of the multiple interactions of scaffolding and signaling proteins within focal adhesions. The manuscript by Gonzalez Wusener and co-workers is attempting to better understand the role played by the ER-associated PTP1B, a tyrosine phosphatase previously implicated in activation of src (de-phosphorylating the inhibitory Y527), and modulating the interaction and activity of paxillin and

FAK respectively. Previous work by the group has shown that PTP1B deficient cells show an altered spreading, migration behavior, exemplified by shortened cycles of lamellar extension and retraction. Since Rac/Rho signaling as well as paxillin and FAK phosphorylation states are coupled to this adhesion generating and turnover machinery, it is obviously highly relevant to understand the potential function of PTP1B. By employing a target trapping mutant of PTP1B, combined with a bi-molecular fluorescence complementation (BiFC), the authors are attempting to get a better understanding and to identify the P-Tyr-residues on the respective target proteins in focal adhesions. Here, specifically paxillin (residues Y31 and Y118), as well as Y925 in FAK are identified as PTP1B targets, potentially relevant for the above mentioned cellular behavior. Unfortunately, by covering both proteins in this manuscript, the analysis is less pertinent, requiring additional control experiments to validate their claims. In addition, the discussion tries to include PTP1B effects during focal adhesion generation, as well as focal adhesion turnover that are not easy to reconcile based one presented data. However, since the PTP1B specific regulation of FAK, could be highly relevant for controlling adhesion dynamics, I would like to suggest to concentrate a bit more on the FAK than on paxillin for a revised version of the manuscript.

Comments for the author

Major issues:
In the introduction to the result section, the role of PTP1B is not carefully explained. For example the cited work by Takino et al (2003) is used to highlight the fact PTP1B overexpression induces downregulation of phosphor-paxillin, which would be perfectly in line with a the proposed role in the manuscript. However, the cited paper actually sees this effect on paxillin, via the de-phosphorylation of crkII, which enhances crkII recruitment to phospho-paxillin and phospho-p130CAS thereby enhancing cell migration. crkII-Y221F is acting dominantly to induce cell migration that can be inverted by DN-Rac1, suggesting that PTP1B has a biological activity mimicking the Y221F-state of crkII. Consistently, PTP1B deletion causes problems in lamellipodial extensions, consistent with a targeting and activation of crkII (by Y221-dephosphorylation). Since crkII has also an SH3-dependent PTP1B binding activity, it would consist of a perfect adapter protein to indirectly bind PTP1B to paxillin in a de-phospho-tyr-dependent manner. While this is all speculation, the recent analysis of the paxillin dissociation dynamics from focal adhesions has clearly shown that P-Y31 and P-Y118 are key for paxillin stabilization in focal adhesions, and their targeting needs to be carefully controlled during the extension reaction cycles of lamellipodia in the periphery of cells (Ripamonti et al., 2021; Communications Biology).

In order to reconcile the actual function of the phosphorylated tyr-residues in paxillin and their targeting by PTP1B, the authors data are very exciting. Apparently, only a small area of the focal adhesions is showing BiFC between PTP1B-DA and paxillin. To fully appreciate this finding a triple labeling for paxillin (or phosphor-paxillin for that matter) would really underscore that only a subpopulation of paxillin may be targeted by PTP1B. Since it is likely that there is an extensive amount of non-overlapping P-pax staining with the BiFC signal (based on Fig S1), the substrate trapping and BiFC interaction with paxillin may be indirect, potentially mediated via crkII, and or FAK.

Please also show BiFC between PTP1B-wt and paxillin. Time lapse imaging could be shown to indicate whether BiFC positive adhesions are sliding, or are stable over time. Does the tethering of the ER-via BiFC/PTP-1B to focal adhesions, influence the turnover of such adhesions. TIRF imaging would be illustrative to detect proximity of the ER-associated PTP1B with the focal adhesion plane. This would also allow to understand whether the BiFC signal from the FAK-3F is localized to the cytoplasm or focal adhesions (see below).

Unfortunately, Fig3 is not allowing conclusive analysis of the BiFC signal in respect to pax-expression as claimed in the quantification. The anti-paxillin staining is not performed, to evaluate the respective expression levels of the two BiFC partners. Instead anti-GFP is used that is detecting both the co-expressed YC-PTP1B, as well as the YN-paxillin. Since the double labeling is not performed, it is not clear whether a reduction of BiFC is simply linked to reduced paxillin transfection. Moreover, for this critical experiment, showing the state of the p-paxillin would be required to conclude whether the absence of BiFC is indeed linked to the phosphorylation state of paxillin, or some other parameter.

Fig4-5: anti-FAK/antiPTP1B/BiFC triple- labeling should be performed to better understand the specific localization of the BiFC signal within focal adhesions. The anti-vinculin is an indication that only a subset of the adhesion is targeted (see above). However, more importantly, FAK-mutants with potentially perturbed focal adhesion-localization, or showing enhanced central/perinuclear BiFC (FAK-3F), should be analysed for the specific localization of the BiFC signal. When the BiFC signal is more centrally localized, a role in FAK recycling or reactivation may be proposed, rather than a function in the focal adhesion.

Fig5: the anti-GFP antibody detects both the transfected FAK and PTP1B protein. This does not allow conclusive ratio-analysis and quantification. Although the shown examples are intriguing, proposing a specific role of Y925 for PTP1B-recognition, the shown examples are not valid, unless it is clear where, and how much FAK is actually present in the transfected cells.

Fig7: please indicate in the legend, what kind of Ko cells are used in this experiment PTP1B. However and much more problematic, since the anti-GFP labeling is not representing the actual amount of transfected paxillin and/or FAK inside these cells, it does not make sense to calculate a BiFC ratio in respect to the GFP-signal. Anti-pax as well as anti-FAK should be revealed independently from the BiFC signal. Triple labeling is required. Here in this experiment a possible difference of FAK-pax BiFC could be linked to either (de)phospho-mimetic pax or FAK-mutations. BiFC interaction can be strongly context dependent. Swapping the fluorophore-fragment from the N-terminus of the proteins to their c-termini should be included as controls. While this might not be helpful in the case of paxillin, where the N-terminus is pointing away from the membrane, and the c-terminus towards the membrane, this situation is different in FAK. In fact, an N-terminal FAK-YN-label, may indeed escape BiFC when FAK is in an active membrane bound conformation (via the FERM domain). On the other hand, an inactive form of FAK, present in the cytoplasm may allow a confirmation in which the N-terminal FERM domain is closer to the c-terminal FAT-domain, carrying the potential Y925-P residue targeted by PTP1B.

The inhibition of BiFC between PTP1B and paxillin by FRNK overexpression does not make sense to me. This FAK-fragment is recruited to focal adhesion bound paxillin that is likely to be normally phosphorylated, although the literature may propose otherwise. Please verify with anti-P-Pax antibodies whether in this specific context of FRNK expression the potential P-pax and respective PTP1B target is absent in these cells. Accordingly, if the paxillin BiFC is due to the recruitment of PTP1B to the P-Y925 of FAK, overexpression of FRNK might also affect the latter interaction in focal adhesions, but potentially not in central-locations in the cell.

Molecular dynamics simulation and helical structure of the Y925-containing peptide: Indeed molecular dynamics analysis can be performed in order to analyse the stability of the FAT domain, for example in response to Y-925 phosphorylation. Based on the potential recognition of this sequence by PTP1B it would even be very relevant to know why the phosphorylated FAT domain can not bind paxillin, while associating easily with PTP1B. Structural information of phosphorylated Tyrosine containing peptides, clearly show that such sequences adopt a beta-sheet structure, which is maintained when bound to an SH2 domain. Indeed, such a beta-sheet locally unwinding helix-1 would likely disrupt the FAT-domain helix bundle, especially when bound to adapter proteins. Thus, considering such additional scenarios, or that of the PTP1B target site, being able to accommodate a p-tyrosine sidechain within a helical conformation could be of high interest to better understand the functions of site-specific phosphatase activities. Maybe a word on the conformation of the active sites of PTP's would be helpful for the readers.

Minor issues:
You could indicate the non-transfected control cells in panel S1B for their endogenous anti-paxillin reactivity.

Merging green with red should be changed to green and magenta (follow guidelines for color-bling readers)

Figure 1 westernblots: The low expression/recognition of FAK-5F and 3F by the anti-FAK antibodies should be clarified. Since anti-GFP is a completely different antibody, its reactivity could be much stronger than that of anti-FAK. Therefore for the anti-GFP blot, at least one additional FAK

construct that is normally recognized by the anti-FAK antibody should be included. If FAK-5F and 3F are much less expressed than the other construct, it is normal that BiFC with PTP-1B is reduced. The type of imaging; e.g. confocal, epifluorescence, TIRF, should also be indicated in the legend of each figure.

Legend to figure S2: legend is not in accordance to panels C and G: the text states anti-GFP to BiFC ratio, but measured was anti-paxillin? Please clarify, and in case anti-GFP was used in combination with BiFC, the quantitative analysis might be meaningless, because you do not know the relationship between both transfected proteins.

First revision

Author response to reviewers' comments

Answer to reviewer 1

Major points
1. The BiFC analysis would benefit from the analysis of another tyrosine phosphorylated adhesion protein that is NOT a target for PTP-1B and thus would presumably not bind the CA mutant.

New data. We have done BiFC experiments using several BiFC pairs which are putative substrates of PTP1B according to a previous proteomic study (Mertins et al, Molec Cell Prot 7: 1763, 2008), including YC/YN-vinculin, a protein which localizes to focal adhesions and is phosphorylated in tyrosine by Src family kinases (Zhang et al, Mol Biol Cell 15: 4234, 2004). Although the vinculin constructs display a subcellular distribution like that of the endogenous protein, we were unable to detect a positive BiFC signal with the substrate trap PTP1B DA. We added representative images in the new Fig S4E,F and a short text in Results (lines 212-214). The average levels of total vinculin in cells transfected with the YC-vinculin construct was about 2-2.5 times of that in non transfected cells (measured by the anti-vinculin staining, not shown). We know that both BiFC constructs are co-expressed in the observed cells because we used a polyclonal anti-GFP which labels both YN and YC fragments with similar intensity and allows the recognition of the different signal distribution of PTP1B (on ER) and vinculin (on adhesions). This is clearly shown in the inset of the new Fig. S4E,F.

2. The analysis is largely unidimensional. Biochemical co-IP expts should be performed to confirm the specificity of the substrate trapping BiFC analysis.

The present BiFC analysis was based on our previous observations showing a) selective colocalization of a GFP-PTP1BDA construct with endogenous paxillin and FAK but not with zyxin within adhesions (Hernández et al, J Cell Sci 119: 1233, 2006), b) significant reduction of PTP1B DA targeting to cell-matrix adhesions in cells lacking paxillin and FAK expression (Burdisso et al, J Cell Sci 126: 1830, 2013). In these studies, we performed immunoprecipitation of PTP1B DA, but we were unable to detect paxillin and FAK in the complexes. Negative co-IPs of functional interacting partners, either related to PTP1B or other PTPs, abound in the literature (for PTP1B see Zhang et al, J Biol Chem 281: 1746, 2006 for alpha actinin; Hughes et al, Mol Biol Cell 26: 3867, 2015 for Mena; Fan et al, J Biol Chem 290: 15934, 2015 for Src; reviewed in Tiganis and Bennett, Biochem J 402: 1, 2007; for PTP-PEST see Shen et al, J Biol Chem 273: 6474, 1998).

Our starting assumption, shared by others (Haj et al, Science 295: 1708, 2002; Yudushkin et al, Science 315: 115, 2007), was that due to the high catalytic rate of PTP1B (Zhang et al, Biochem 33: 2285, 1994), the steady state concentration of wild type PTP1B-substrate complexes in cell-matrix adhesion sites would represent a small fraction of total PTP1B and substrates. The substrate trap mutant (DA) of PTP1B is expected to increase the concentration of enzyme-substrate complexes by dramatically reducing the catalysis without affecting substrate binding (Flint et al, PNAS 94: 1680, 1997). Of note, in intact cells, PTP1B and its putative substrates at adhesions are in different membrane compartments (ER and PM, respectively). ER extends dynamically to the cell periphery in a microtubule-dependent manner (Waterman-Storer and Salmon, Curr Biol 8: 798, 1998; Santama et al, J Cell Sci 117: 4537, 2004; Fuentes and Arregui, Mol Biol Cell 20: 1878, 2009), increasing the

probability of ER-PM interactions (Zhang et al, J Cell Sci 123: 3901, 2010; Hernández et al, J Cell Sci 119: 1233; Burdisso et al, J Cell Sci 126: 1830, 2013). By time lapse and TIRFM we have shown that BiFC puncta formed between ER-bound PTP1B DA and plasma membrane-associated Src occur at the distal tips of ER tubules "dipping down" in the fluorescent evanescent field close to the membrane/substrate interface (Monteleone et al, Plos One 7: e38948, 2012), consistent with similar observations of microtubules-adhesion contacts (Krylyshkina et al, J Cell Biol 161: 853, 2003). Thus, the fraction of PTP1B DA forming complexes with either phosphorylated FAK or paxillin at cell-matrix adhesions is expected to be small and spatially restricted, both conditions for BiFC is suitable.

The specificity of the substrate trapping BiFC analysis was confirmed by: 1) the significant reduction of the BiFC fluorescence in cells treated with vanadate, a competitive inhibitor that reduces the binding of the PTP1B DA for its substrates, 2) the undetectable BiFC with the wild type PTP1B in comparison with the substrate trap mutant, and 3) the substantial reduction of the substrate trap BiFC by selective point mutations of the phospho-acceptor tyrosine residues in Src (Monteleone et al Plos One 7: e38948, 2012), alpha actinin (Burdisso et al., J Cell Sci 126: 1830, 2013) in which we confirmed that the target site was the tyrosine-12 (unpublished results), and paxillin and FAK (present manuscript). Altogether we consider that combining BiFC with the substrate trap approach provides superior sensitivity to detect PTP1B-substrate interactions in their natural sub-cellular compartments. In addition, BiFC combined with microscopy allows for a selective quantitative analysis of PPIs in adhesions, providing information of the magnitude and range of the signal, as well as its spatial distribution on individual adhesions and cells. This information is not directly available on co-IPs.

3. PY blotting of the tagged pxn and FAK WT and mutant constructs should be performed from WT and KO PTP-1B cells. This would help further validate the proposed specificity of the FAK PY925 data, which is currently rather weak.

New data. We have not performed phosphotyrosine staining of paxillin and FAK in WBs for this work. However, in our previous publications (Arregui et al, J Cell Biol 143: 861, 1998; González Wusener et al, Biol Open 5: 32, 2016) we have examined this issue and found that paxillin and FAK have reduced phosphotyrosine in PTP1B KO cells compared to KO cells reconstituted with PTP1B. These results argue against a direct and primary role of PTP1B on the dephosphorylation of bulk FAK and paxillin. Instead, PTP1B is a prominent activator of the tyrosine kinase Src, which then together with FAK phosphorylate FAK and paxillin (PTP1B ▫ Src/FAK ▫ paxillin/p130Cas….). This role of PTP1B as an activator of Src has been extensively confirmed by many groups (including ours) and in several cell types (Bjorge et al., 2000; Cheng et al., 2001; Arias-Salgado et al., 2005; Liang et al., 2005; Anderie et al., 2007; Zhu et al, 2007; Cortesio et al., 2008; Monteleone et al., 2012; Fan et al., 2015; González Wusener et al., 2016; Mei et al., 2016; Song et al., 2016; Faria et al., 2019).

The design of the present BiFC study provided new information suggesting that in addition to its role as activator of Src, PTP1B directly dephosphorylates FAK and paxillin in cell-matrix adhesions. Regarding the concern of the reviewer about the specificity of the BiFC signal in the FAK pY925 residue, it should be noted that the BiFC signal of the DA/FAK pair is significantly reduced when the DA/FAK Y925F mutant was used (Fig. 5M-O). Thus, the substrate trap DA must bind to phosphorylated Y925 in the FAK construct. The link between pY925 and PTP1B can be better visualized in individual PTP1B KO cells reconstituted with GFP-PTP1B or GFP-PTP1B CS, an inactive mutant in which the catalytically essential cysteine 215 is replaced by serine (Guan and Dixon, J Biol Chem 266: 17026, 1991). Whereas cells expressing wild type PTP1B display positive anti-FAK pY925 signal in adhesions, non transfected cells or cells expressing the catalytically inactive (CS) mutant showed background levels (new Fig. 6B-G). These results indicate that FAK Y925 phosphorylation requires PTP1B activity. Alternatively, mouse embryonic fibroblasts transfected with the CS, which has a dominant negative effect on endogenous PTP1B (Arregui et al., J Cell Biol 143: 861, 1998) show reduced pY925 signal compared to non transfected cells (new Fig. 6H-J). These results further reinforce the idea that PTP1B plays a major role in the phosphorylation of FAK Y925, likely through Src activation. The phosphorylation of FAK Y925 requires Src activity (Brunton et al., Cancer Res 65: 1335, 2005). We confirmed that SYF cells lacking Src activity (triple knockout of Src, Yes and Fyn) have low if any, phosphorylation of FAK Y925, and that expression of constitutive active Src in these cells leads to robust pY925 signal in focal adhesions. We added these data in the new Fig. 6K-L. Additional text related to this topic was added in Results, between

lines 292-308 of the manuscript.

4. Can the authors explain why the BIFC labeling of focal adhesions is more robust and more focal adhesion-like (as opposed to punctate) with the FAK WT, Y397F, 3F mutants (fig 5C-H)?

New data. Figure 5 shows the BiFC analysis of PTP1B-FAK complexes. Representative panels of paired signals for GFP and BiFC are shown to visualize the magnitude and distribution of signals. The polyclonal anti-GFP used in this work labels both YN and YC fragments with similar intensity, and the recognition of the different signal distribution of PTP1B (on ER) and FAK (on adhesions) reveals that both BiFC pairs are expressed in the same cell (new Fig. S1). As the reviewer noted, the BiFC channel shows a robust labeling of adhesions with FAK WT, and the Y397F, and 3F mutants (fig 5C-H). The distribution of BiFC in individual adhesions is most frequently visualized as bright punctate, but a wider distribution (as pointed by arrowheads in panels E and K of Fig. 4) can also be observed. The mechanisms controlling the distribution of the BiFC label within individual adhesions are presently unknown. The magnitude and distribution of the BiFC label could be conditioned by several factors, including: 1) the angles in which peripheral ER tubules approach to the plasma membrane and contact the substrates, 2) the spatial distribution and mobility of the phosphorylated substrates in adhesions, 3) the dynamics of the peripheral ER and PM contacts, and 4) the density of spots. Ballestrem et al (J Cell Sci 119: 866, 2006) has described “hotspots” of phosphorylated paxillin and FAK within adhesions, the functional significance of which are unknown. We confirmed the non uniform distribution and “hotspots” of phospho-paxillin in adhesions in the new Fig. S3. A high-resolution time-series that nicely illustrates the dynamic of peripheral ER puncta over adhesions can be seen in a previous paper from our lab (Fig. 4D and 6A, Hernández et al, J Cell Sci 119: 1233, 2006). We have tried to analyze the dynamic of the BiFC label in living cells, but the fast photobleaching of EYFP fluorescence precluded the acquisition of satisfactory time-series with our current microscope settings. Still, short-term videos showed movement and fusions of puncta over adhesions, likely reflecting ER-PM membrane contacts, as discussed previously (Arregui et al., Cell adhesion & Migr 7: 418, 2013).

5. The modeling of electrostatic effects on FAK conformation doesn’t really add anything to the dataset and largely confirms already published experimental data.

Fig. 8 of the manuscript shows the results of computational simulations to analyze the relevance of the phosphorylation state of FAK Y925 on electrostatic effects that could have an impact on the conformation of the FAT domain. Panels in Fig. 8A highlight the electrostatic surfaces of each individual helix in the context of the 4-helix bundle, underscoring the hydrophobic nature of the internal faces and the charged and polar characteristics of the external surfaces. As the reviewer noted, this may not be necessary, and we agree to remove this panel from the figure. Panels B and C; however, contribute directly to the dataset as follows: Panel B shows details of relevant charged residues in helices 1, 2 and 4 that engage in electrostatic interactions when helix 1 is not docked to the bundle, and thus contribute to the reclosure of the 4-helix bundle. Panel C shows the last frame of a simulation starting from the open conformation of the FAT domain, either phosphorylated or not phosphorylated in the Y925, and shows the clear difference—in terms of electric field—of the interactions between helices 1 and 2; there it is observed that the non-phosphorylated case shows strong attractive interactions among the residues shown in panel B, while the phosphorylated case does not. To the best of our knowledge, this electrostatic effect has not been previously reported.

In the structural analysis of the FAT domain, Arold et al, Structure 10: 219, 2002, pointed out two salt bridges between helix 1 and helix 4 (R919*h1* with D1039*h4* and D922*h1* with R1042*h4*) as contributing to the anchorage of helix 1 to the bundle. This study does not mention the electrostatic interactions between K923 of the helix 1 and the E970 of the helix 2 emerging from our simulations starting from the open FAT conformation. Other publications of FAT structures (Hayashi et al, Nature Struct Biol 9: 101, 2002; Kadaré et al, J Biol Chem 290: 479, 2015) also omit to mention the electrostatic interactions between helices 1 and 2. Of note, we did not find the electrostatic interactions between helix 1 and 4 described by Arold et al. The reason for this is that we started the simulation from the open FAT conformation since this is the state in which Y925 is most likely phosphorylated (Arold et al, Structure 10: 219, 2002; Prutzman et al, Structure 12: 881, 2004) and as such, accessible to PTP1B. After dephosphorylation, it is expected that helix 1 would restore the anchorage to the rest of the bundle. Our simulations did not reach that final closed

conformation (presumably they were not long enough), thus failing to show the interactions found by Arold et al. But contrary to this being a problem, it is just a welcomed observation that sheds light on the way the phosphorylated and non-phosphorylated systems differ in terms of long-range interactions that guide their behavior towards the final conformation. To the best of our knowledge this type of analysis has not been published before and provides a physical, atomistic view of how the dephosphorylation of FAK Y925 by PTP1B at a molecular scale may impact on the stabilization of the FAT conformer to mediate interaction with paxillin.

6. The Discussion is overly detailed and speculative, and largely unsupported by new experimental data (eg. regulation of downstream signaling from Y925). It could be shortened considerably, and/or supported by additional experimentation.

The Discussion was revised and shortened to reduce the speculative statements.

Minor points

1. As noted in the discussion, PTP-PEST is another major phosphatase that regulates focal adhesions and it, like FAK also binds pxn (Shen JBC 1998, Cote et al JBC 1999,) and regulates pxn and Rac1 signaling (Jamieson JCS 2005). Discussion of the respective roles for PTP-PEST vs PTP-1B could be expanded to address their respective roles.

We thank the reviewer for raising this point which we overlooked. In response to the suggestion, we added new paragraphs in Discussion (lines 453-465).

2. Is there any pxn in focal adhesions in the FAK KO cells? Could this explain the lack of a BiFC signal (Fig 3 K,L)? There also seems to be more a-GFP spots in this image than suggested by the quantitation (Fig 3M).

Yes, immunolabeling of endogenous paxillin as well as transfected YN-paxillin (detected with a-GFP) colocalize with a-vinculin-labeled adhesions in FAK KO cells. The scattered a-GFP and BiFC spots that are seen in FAK KO cells could be due to compensating expression of the FAK-related Pyk2, which has a punctate distribution in the cytoplasm (Schaller and Sasaki, J Biol Chem 272: 25319, 1997; Sieg et al, EMBO J 17: 5933, 1998; Du et al, J Cell Sci 114: 2977, 2001), and still can phosphorylate cytoplasmic paxillin (Richardson and Parsons, Nature 380: 538, 1996; Tilghman et al J, Cell Sci 118: 2613, 2005; Lim et al J Cell Biol 180: 187, 2008). Our quantification of BiFC/GFP ratios in Fig. 3M was done on segmented adhesion complexes revealed by vinculin or paxillin, where the residual a-GFP and BiFC puncta do not accumulate.

3. Does the positioning of the BiFC constructs on opposite ends of the relevant molecules impact outcomes?

A priori, the rationale for adding the BiFC construct to the N- or C-terminus of the protein of interest was based on previous empirical evidence using tagged versions of the proteins which demonstrate no interference with localization, phosphorylation, interactions, and other functions. C- fusions of PTP1B impair ER targeting so we only use N-fusions, as reported for full length fluorescent proteins (Arregui et al., J Cell Biol 143: 861, 1998; Haj et al Science 295: 1708, 2002; Takino et al, J Cell Sci 116: 3145, 2002).

Paxillin is an adaptor protein with protein-interacting modules at the N- and C-terminus. We received the GFP-paxillin construct (GFP at the N-terminus of paxillin) from Kenneth Yamada (NIH). From there we made the BiFC constructs, either with the YN or YC fragment added to the N-terminus of paxillin. We, like others, have demonstrated that these tagged paxillin constructs target to adhesions similar to the endogenous proteins. Some relevant papers using N-terminal fusions are: (Zamir et al, Nature Cell Biol 2, 191, 2000; Takino et al, Oncogene 21: 6488, 2002; von Wichert et al, EMBO J 22: 5023, 2003; Ballestrem et al J Cell Sci 119: 866, 2006; Hernández et al, J Cell Sci 119: 1233, 2006). Our present results (Fig. 7) show that interactions between FAK and N-terminus YN-paxillin is not prevented. We are aware that C-terminus tagged paxillin was successfully used to study adhesion dynamics (i.e. Webb et al Nature Cell Biol 2004) and paxillin recruitment mechanisms to adhesions (i.e. Ripamonti et al Commun Biol 2021). However, we did not analyze paxillin constructs with YC/YN in the C-terminus.

For FAK constructs we used the GFP-FAK construct from Thomas Parsons (U. Virginia) as template. We decided to go for N-terminal fusions of FAK as there is plenty of literature using these. In addition, FAK tagged at the C-terminus was unable to bind Grb2, a known binding partner of the FAT domain (Schlaepfer et al Nature 372: 786, 1994; Cary et al., J Cell Biol 140: 211, 1998). Some relevant papers using N-terminus-tagged FAK are: (Webb et al Nature Cell Biol 2: 154, 2004; Tilghman et al, J Cell Sci 118: 2613, 2005; Hernández et al, J Cell Sci 119: 1233, 2006; Cai et al, Mol Cell Biol 28: 201, 2008). For the reasons indicated above we did not analyze C-terminus fusions of FAK.

Answer to reviewer 2

Major points

1) expression levels of the constructs need to be validated. This is very convincingly done for paxillin in supplemental figure 1. This should also be shown for the paxillin mutants, wild type FAK and its mutants and the PTP1B constructs. The authors appear to have the data and this should be shown in additional supplemental figures. This is important to rule out effects due to overexpression of exogenous constructs.

New data. We show the relative levels of expression of exogenous BiFC constructs and endogenous paxillin and FAK in the Western blots of the Figure 1B. Only YN-5F and YN-3F FAK constructs could not be detected with the anti-FAK used, likely because in these constructs the mutations are localized within the region used as immunogen. As the reviewer noted, we also analyzed the levels of expression of the YN-paxillin construct in individual cells by immunofluorescence (original Fig. S1). In the new manuscript, we further added quantitative analysis of the expression levels of YN-pax Y31/118 mutants, and also of YN-FAK and YN-FAK Y925F, which are the most relevant constructions related to the results and conclusions (new Fig. S2). In addition, we monitored the expression levels of the BiFC constructs using anti-GFP. Note that the polyclonal anti-GFP detects both YN and YC fragments similarly, and this fact allows to verify the simultaneous co-expression of BiFC constructs by the recognition of the different signal distribution of PTP1B (on ER) and paxillin or FAK (on adhesions). This could be appreciated in the new Fig. S1. For the comparative analysis shown in Figs. 3 and 5, the acquisition conditions of images were setup using the BiFC produced by PTP1B DA/paxillin and PTP1B DA/FAK pairs, which represent the positive conditions, and maintained for all the other experimental conditions. We exclude from the analysis images with saturated pixels.

2) the BiFC signal is not uniformly distributed in the adhesions. How does this compare to paxillin distribution and phosphorylated paxillin distribution? Have the authors looked at this or is it documented in the literature. Related, the BiFC signal between PTP1B and FAK appears different in distribution in adhesions that the BiFA signal between PTP1B and paxillin. Are there really differences?

New data. The appreciation of the reviewer is correct, BiFC signal in most cases for paxillin and FAK localizes in a punctate pattern, with a single punctum, or a few puncta within the area stained by the adhesion marker (paxillin or vinculin). As mentioned in the response of Reviewer 1, point 4, the mechanisms controlling the distribution of the BiFC label within individual adhesions are unknown. The BiFC label could be conditioned by several factors, including the angles in which peripheral ER tubules approach to the plasma membrane and contact the substrates, by the spatial distribution and mobility of the phosphorylated substrates within adhesions, the dynamics of the ER, and the density of spots. Several works have shown non uniform distribution of phosphorylated paxillin within adhesions (Zamir et al J Cell Sci 112: 1655, 1999; Zaidel-Bar et al J Cell Sci 120: 137, 2006) and FAK (Ballestrem et al J Cell Sci 119: 866, 2006), the functional significance of which is unknown. We confirmed the non uniform distribution and "hotspots" of phospho-paxillin in adhesions in the new Fig. S3. A high-resolution time-series that nicely illustrate the development and movement of peripheral ER puncta over adhesions can be seen in a previous paper from our lab (Fig. 4D and 6A, Hernández et al, J Cell Sci 119: 1233, 2006). We have tried to analyze the dynamic of the BiFC label in living cells, but the fast photobleaching of EYFP fluorescence precluded the acquisition of satisfactory time-series with our current microscope settings. Still, short-term videos

show movement and fusions of puncta over adhesions, likely reflecting ER-PM membrane contacts (Arregui et al., Cell adhesion & Migr 7: 418, 2013). At the present time we have no convincing evidence to affirm that BiFC signal distributions from DA/paxillin and DA/FAK are different. They both look punctate, and in some cases the label extends as a short segment, that colocalizes with ER tubules, where PTP1B is located.

3) BiFC in figures 2 and 4 is quite sharp and prominent in adhesions. In figure 3 and 5 there is more diffuse background staining. Why?

Images in Figures 2 and 4 were background-subtracted using a rolling ball algorithm (Sternberg, Computer 16: 22, 1983) to flatten the image and then sharpened using "unsharp mask" from Image J to improve the signal/noise ratio and facilitate the visualization of the ER. This procedure was not applied in Figs 3 and 5 since the goal is not to show small details in the signal distribution but to display the general pattern and the intensity. In this case, an extracellular ROI was used to substract the background from the images and then a slight bright/contrast adjustment was applied to all images. We will add this information in the legend of the figures.

4) Supplemental figure 3 needs a control of cells in suspension, or other negative control, to validate adhesion dependent phosphorylation of Erk in their assay.

New data. We agree with the reviewer and added the requested data in the new Fig. S7.

5) The PTP1B KO cells show elevated interactions between FAK and paxillin that the authors ascribe to altered phosphorylation of Y925 on FAK and a change in the FAT domain conformation. This is not consistent with the modest, insignificant increase in phosphorylation of this site determined by blotting. This would also require high stoichiometry of phosphorylation. Without this additional data, this conclusion is not strongly supported by the evidence. The observation is intriguing, but the authors need to consider and discuss other potential mechanisms as well, and these could be indirect.

New data. If we understand correctly, the reviewer refers to data in Figure 6, where we analyzed the phosphorylation state of FAK Y925 in PTP1B WT and KO cells (plus/minus Src overexpression), and in Figure 7, where we analyzed the potential consequences of the phosphorylation state of FAK Y925 on paxillin-FAK interactions. Just as a reminder, the biochemical and structural data indicate that phosphorylated Y925 occurs in the open conformation of the FAT domain, state that is incompatible with paxillin binding. Conversely, non phosphorylated Y925 is present in the closed conformation of the FAT domain, and this state facilitates paxillin binding. Since the BiFC results shown in Fig. 5 implicate that pY925 is a target for PTP1B dephosphorylation, we decided to analyze the phosphorylation status of this residue in PTP1B WT and knockout cells. In the original Fig. 6 we showed the Western blots analysis using a phospho-specific antibody. Results from three experiments did not show significant differences between KO and WT cells. As PTP1B is required to activate Src (Arregui et al., 1998; Bjorge et al., 2000; Cheng et al., 2001; Arias-Salgado et al., 2005; Liang et al., 2005; Anderie et al., 2007; Zhu et al, 2007; Cortesio et al., 2008; Monteleone et al., 2012; Fan et al., 2015; González Wusener et al., 2016; Mei et al., 2016; Song et al., 2016; Faria et al., 2019), and Src is the main kinase phosphorylating FAK Y925 (PTP1B □ □Src □ □FAK pY925), the phosphorylation status of FAK Y925 is in part the result of the relative activities of PTP1B and Src. Indeed, the overexpression of constitutive active Src in PTP1B knockout cells increases significantly the pY925 signal (original Fig. 6). In the new Fig. 6 we provided additional data supporting the dual requirement of PTP1B and Src in the phosphorylation status of FAK Y925. PTP1B also has a dual role. One is to activate Src and ensure the phosphorylation of FAK Y925 (new Fig. 6B-M). a second role, which is unraveled by our BiFC analysis, is the direct regulation of this site (PTP1B □ □ FAK pY925).

Minor points
1) the Flint reference and the description of pervanadate specifically blocking substrate trapping needs to appear in the results section when the experiment is first described.

Done.

2) what does the anti-GFP antibody recognize? YN, YC or both?

The polyclonal anti-GFP used in our work recognizes both YN and YC fragments with similar intensity. We have evaluated this in cells expressing equivalent levels of YC-FAK or YN-FAK constructs (assessed by anti-FAK). This antibody was raised against the full GFP protein (Invitrogen, catalog A6455).

3) the authors should use the word "doubled" rather than "duplicated" to describe the level of expression of the exogenous constructs.

Corrected in the text.

Answer to reviewer 3

Major issues:
1. In the introduction to the result section, the role of PTP1B is not carefully explained. For example the cited work by Takino et al (2003) is used to highlight the fact PTP1B overexpression induces downregulation of phosphor-paxillin, which would be perfectly in line with a the proposed role in the manuscript. However, the cited paper actually sees this effect on paxillin, via the de-phosphorylation of crkII, which enhances crkII recruitment to phospho-paxillin and phospho-p130CAS thereby enhancing cell migration. crkII-Y221F is acting dominantly to induce cell migration that can be inverted by DN-Rac1, suggesting that PTP1B has a biological activity mimicking the Y221F-state of crkII. Consistently, PTP1B deletion causes problems in lamellipodial extensions, consistent with a targeting and activation of crkII (by Y221-dephosphorylation). Since crkII has also an SH3-dependent PTP1B binding activity, it would consist of a perfect adapter protein to indirectly bind PTP1B to paxillin in a de-phospho-tyr-dependent manner. While this is all speculation, the recent analysis of the paxillin dissociation dynamics from focal adhesions has clearly shown that P-Y31 and P-Y118 are key for paxillin stabilization in focal adhesions, and their targeting needs to be carefully controlled during the extension reaction cycles of lamellipodia in the periphery of cells (Ripamonti et al., 2021; Communications Biology).

In order to reconcile the actual function of the phosphorylated tyr-residues in paxillin and their targeting by PTP1B, the authors data are very exciting. Apparently, only a small area of the focal adhesions is showing BiFC between PTP1B-DA and paxillin. To fully appreciate this finding a triple labeling for paxillin (or phosphor-paxillin for that matter) would really underscore that only a subpopulation of paxillin may be targeted by PTP1B. Since it is likely that there is an extensive amount of non-overlapping P-pax staining with the BiFC signal (based on Fig S1), the substrate trapping and BiFC interaction with paxillin may be indirect, potentially mediated via crkII, and or FAK.

New data. We referred to the results shown in the paper of Takino et al without interpreting it. Regarding the considerations made by the reviewer on CrkII, as a potential adaptor recruiting PTP1B to paxillin in an SH3-dependent manner, we performed BiFC experiments using a proline mutant of PTP1B which is deficient in Crk SH3 binding (Liu et al, J Biol Chem 271: 31290, 1996; Dadke and Chernoff, Biochem J 364: 377, 2002; Hernández et al, J Cell Sci 119: 1233, 2006). We find that the magnitude of the BiFC signal in the double mutant DA-PA is similar to that seen in the PTP1B DA-paxillin complexes, arguing against the role of polyproline-SH3 interactions on the BiFC production. We decided not to add these data on the manuscript to avoid confusion but in response to the point raised by the reviewer we added these data in the new Fig. S4 and a short text in Results, lines 204-210.

Regarding the small area showing BiFC of YN-paxillin and YC-PTP1B DA within adhesions, we did staining with anti-paxillin. We observed that the distribution of BiFC signal partly colocalizes with the distribution of anti-paxillin, which recognizes both, the endogenous and exogenous pools. Quantitatively, the areas of bright BiFC puncta coincide with higher anti-paxillin intensity levels. The area of the adhesion extending beyond the BiFC signal has comparatively lower anti-paxillin intensity. In experiments with cells expressing YN-paxillin alone (without YC-PTP1B DA expression, thus no BiFC), the distribution of the construct, detected by anti-GFP colocalizes tightly with endogenous vinculin, as previously reported (Zamir et al, J Cell Sci 112: 1655, 1999; Humphries et al J Cell Biol 179: 1043, 2007; Digman et al, Biophys J 96: 707, 2009). Thus, we think that

complexes induced by the substrate trap YC-PTP1B DA led to some redistribution of YN-paxillin in BiFC spots, likely due to mobile pools of paxillin within adhesions (Digman et al, Biophys J 94: 2819, 2008; Choi et al, Biochem J 100: 583, 2011; Legerstee et al, Sci Rep 9: 10460, 2019). As mentioned in our response to R2, point 2) the mechanisms controlling the distribution of the BiFC label within individual adhesions are unknown. The BiFC label could be conditioned by several factors, including the angles in which peripheral ER tubules approach to the plasma membrane and contact the substrates, by the spatial distribution and mobility of the phosphorylated

substrates within adhesions, the dynamics of the ER, and the density of spots. Ballestrem et al (J Cell Sci 119: 866, 2006) has described "hotspots" of phosphorylated paxillin and FAK within adhesions, the functional significance of which are unknown. We confirmed the non uniform distribution and "hotspots" of phospho-paxillin in adhesions in the new Fig. S3. A high-resolution time-series that nicely illustrate the development and movement of peripheral ER puncta over adhesions can be seen in a previous paper from our lab (Fig. 4D and 6A, Hernández et al, J Cell Sci 119: 1233, 2006).

2. Please also show BiFC between PTP1B-wt and paxillin. Time lapse imaging could be shown to indicate whether BiFC positive adhesions are sliding, or are stable over time. Does the tethering of the ER-via BiFC/PTP-1B to focal adhesions, influence the turnover of such adhesions. TIRF imaging would be illustrative to detect proximity of the ER-associated PTP1B with the focal adhesion plane. This would also allow to understand whether the BiFC signal from the FAK-3F is localized to the cytoplasm or focal adhesions (see below).

We show the BiFC between PTP1B WT and paxillin in Fig. 3B, and the quantification of peripheral BiFC/anti-GFP signal ratio in 3M. PTP1B WT did not produce BiFC, likely due to the high catalytic rate of PTP1B (Zhang et al, Biochem 33: 2285, 1994) and the small steady state concentration of wild type PTP1B-substrate complexes in cell-matrix adhesion sites compared to the total PTP1B and substrates. The substrate trap mutant (D/A) of PTP1B is expected to increase the concentration of these complexes by dramatically reducing the catalysis without affecting the ability to bind substrates (Flint et al, PNAS 94: 1680, 1997; Haj et al, Science 295: 1708, 2002; Yudushkin et al, Science 315: 115, 2007).

As mentioned above, we did time-lapse of the substrate trap fused to GFP. This forms bright puncta that frequently slide over adhesions (Fig. 4D and 6A, Hernández et al, J Cell Sci 119: 1233, 2006). We have tried to analyze the dynamic of the BiFC label in living cells, but the fast photobleaching of EYFP fluorescence precluded the acquisition of satisfactory time-series with our current microscope settings. Still, short-term videos show apparently saltatory movements and fusions of puncta over stationary adhesions, likely reflecting ER-PM membrane contacts (see model at Arregui et al., Cell adhesion & Migr 7: 418, 2013).

In a previous study from our laboratory (Burdisso et al J Cell Sci 126: 1820, 2013) we show that in PTP1B KO cells transfected with wild type PTP1B, peripheral adhesions targeted with ER tubules have lifetimes ranging 11-36 min, with a median of 20 min, while non targeted adhesions or adhesions targeted with ER tubules containing a catalytically inactive PTP1B range 1-12 min, with a median of 4 min. This is consistent with the growth in size of peripheral adhesions targeted by ER in HeLa cells (Zhang et al J Cell Sci 123: 3901, 2010).

3. Unfortunately, Fig3 is not allowing conclusive analysis of the BiFC signal in respect to pax-expression as claimed in the quantification. The anti-paxillin staining is not performed, to evaluate the respective expression levels of the two BiFC partners. Instead, anti-GFP is used that is detecting both the co-expressed YC-PTP1B, as well as the YN-paxillin. Since the double labeling is not performed, it is not clear whether a reduction of BiFC is simply linked to reduced paxillin transfection. Moreover, for this critical experiment, showing the state of the p-paxillin would be required to conclude whether the absence of BiFC is indeed linked to the phosphorylation state of paxillin, or some other parameter.

New data. As mentioned above, in parallel experiments we did staining with anti-paxillin in cells co-expressing YN-paxillin and YC-PTP1B DA and exhibiting BiFC signal. The results from these experiments do not change the conclusions of the results presented in the paper. We chose to

monitor the levels of expression of the BiFC constructs using anti-GFP since the polyclonal anti-GFP labels both YN and YC fragments with similar intensity, and the recognition of the different signal distribution of PTP1B (on ER) and FAK (on adhesions) easily reveals that both BiFC pairs are expressed in the same cell. This could be appreciated in the new Fig. S1.

Regarding the phosphorylation state of paxillin in the different conditions where we quantified the BiFC of paxillin with PTP1B. First, for the BiFC analysis we used PTP1B WT cells, which are a fibroblast cell line expressing endogenous wild type PTP1B and paxillin, and paxillin is tyrosine phosphorylated in adhesions in Y118 (Shown by the first author paper, Gonzalez Wusener et al Biol Open 5: 32, 2016). Since the pair PTP1B DA/pax WT produces a positive BiFC signal, and this is inhibited by the competitive inhibitor pervanadate, the background BiFC in the pair PTP1B WT/pax WT strongly indicates that the BiFC signal requires of the substrate trap mutation Asp□Ala (DA) at the catalytic center of the enzyme (nicely reviewed by Tiganis & Bennet Biochem J 402: 1, 2007). In all these conditions the differences in the BiFC signal are not dependent on the phosphotyrosine levels of the substrate but to the binding and catalytic rate of the enzyme, as demonstrated by many others (Flint et al PNAS 94: 1680, 1997; Haj et al, Science 295: 1708, 2002; Yudushkin et al, Science 315: 115, 2007). PTP1B has a high catalytic rate (Zhang et al, Biochem 33: 2285, 1994), and the steady state concentration of wild type PTP1B-substrate complexes in cell- matrix adhesion sites would represent a small fraction of total PTP1B and substrates. As mentioned above, the substrate trap mutant increases the concentration of these complexes. In cells co- expressing FRNK and in FAK KO cells it is expected that paxillin phosphorylation is severely reduced as reported (Richardson and Parsons, Nature 380: 538, 1996; Tilghman et al J Cell Sci 118: 2613, 2005; Lim et al J Cell Biol 180: 187, 2008). We have shown in Fig. 3 that impairing FAK function reduces the BiFC signal of YN-paxillin/YC-PTP1B DA. We added a new Fig S6 to show the reduction of anti-pY118 signal under these conditions.

4. Fig4-5: anti-FAK/antiPTP1B/BiFC triple- labeling should be performed to better understand the specific localization of the BiFC signal within focal adhesions. The anti-vinculin is an indication that only a subset of the adhesion is targeted (see above). However, more importantly, FAK- mutants with potentially perturbed focal adhesion-localization, or showing enhanced central/perinuclear BiFC (FAK-3F), should be analysed for the specific localization of the BiFC signal. When the BiFC signal is more centrally localized, a role in FAK recycling or reactivation may be proposed, rather than a function in the focal adhesion.

All BiFC mutants of FAK analyzed in Fig. 5 prominently accumulate in peripheral adhesions, although some diffuse intracellular background and punctate is visible, as it occurs with the wild type FAK BiFC construct. The distribution of WT and mutant forms is shown in Fig. 1C of the manuscript. The BiFC analysis in this section aimed to test whether PTP1B is capable of recognizing FAK as a substrate in adhesions. The collective findings that: 1) the substrate trap DA produced BiFC, 2) it is inhibited by the competitive inhibitor pervanadate, and 3) BiFC is not produced when the WT enzyme is used, strongly suggest that the interaction involves the catalytic center of the enzyme and a phosphotyrosine residue in the FAK substrate and argue against scaffold-type interactions. Thus, the mutational analysis on FAK was further designed to identify the target phosphotyrosine within FAK. Since all mutants localize similarly to adhesions, the differential loss of BiFC in FAK mutants containing the Y925, confirmed by the single substitution Y925F, reveals that this is the target residue. We did not study the BiFC signal outside adhesions, in part because the present work builds on previous research from our laboratory suggesting a local role of PTP1B on adhesion regulation (Hernández et al, 2006; Burdisso et al., 2013; González et al., 2016) and the new results identifying FAK as a substrate in adhesions extend nicely to that work. By optical confocal sectioning we have observed a fraction of BiFC puncta located at more central regions in the cell, corresponding to deeper planes away from the cell-substrate interphase, and consistent with endosomal localization, as reported (Alanko et al, Nature Cell Biol 17: 1412, 2015; Nader et al, Nature Cell Biol 18: 491, 2016). The role of FAK in endosomes seems to depend on intact Y397, which is not target of PTP1B according to our results.

5. Fig5: the anti-GFP antibody detects both the transfected FAK and PTP1B protein. This does not allow conclusive ratio-analysis and quantification. Although the shown examples are intriguing, proposing a specific role of Y925 for PTP1B-recognition, the shown examples are not valid, unless it is clear where, and how much FAK is actually present in the transfected cells.

New data. The reviewer is correct, the polyclonal anti-GFP detects both YN/YC fragments in the BiFC pairs. In experiments in which we transfected cells with single YN/YC-FAK (not shown), the anti-GFP labels these constructs with similar intensity when expressed at equivalent levels of total FAK (assessed by anti-FAK in individual cells). This a-GFP antibody was raised against the full GFP protein (Invitrogen, catalog A6455).

We are aware that the best scenario for quantification and comparison would be to have invariant co-expression ratios of the paired YN/YC fusion proteins in all the conditions analyzed. The individual YN/YC constructs are not fluorescent by themselves, and for this reason transfected cells cannot be sorted before analysis. Since: 1) we know the anti-GFP labels similarly both YN/YC fragments, 2) we performed the analysis in a selected cell population co-expressing both constructs, as assessed by a-GFP detection and by recognition of its different subcellular distribution, 3) both YN/YC constructs use the same regulatory sequences for expression (because the base vector is the original pEGFP-C1 plasmid from Clontech), 4) both plasmids encoding YN/YC constructs were transfected in equimolar concentrations, we assumed that cell-cell variations due to the expression levels of the constructs would similarly occur for all constructs, without any particular bias. In the new Fig. S1 we monitored the expression levels of the BiFC constructs using anti-GFP. Note that the polyclonal anti-GFP detects both YN and YC fragments similarly, and this fact allows to verify the simultaneous co-expression of BiFC constructs by recognition of the different signal distribution of PTP1B (on ER) and paxillin or FAK (on adhesions).

6. Fig7: please indicate in the legend, what kind of Ko cells are used in this experiment PTP1B. However and much more problematic, since the anti-GFP labeling is not representing the actual amount of transfected paxillin and/or FAK inside these cells, it does not make sense to calculate a BiFC ratio in respect to the GFP-signal. Anti-pax as well as anti-FAK should be revealed independently from the BiFC signal. Triple labeling is required. Here in this experiment a possible difference of FAK-pax BiFC could be linked to either (de)phospho-mimetic pax or FAK-mutations.

As suggested by the reviewer, we indicated that we used PTP1B WT and PTP1B KO cells. It is true that the anti-GFP does not inform about the levels of the individual YN-paxillin and YC-FAK constructs but the sum of both. Since in cells co-transfected with GFP-FAK and mRFP-paxillin (in the same Clontech C1-based plasmids as used for the YC-FAK and YN-paxillin constructs) we observed co-expression in more than 90% of the transfected cells, we are confident that both plasmids used in the study of Fig. 7 are co-transfected in similar percentages.

7. BiFC interaction can be strongly context dependent. Swapping the fluorophore-fragment from the N-terminus of the proteins to their c-termini should be included as controls. While this might not be helpful in the case of paxillin, where the N-terminus is pointing away from the membrane, and the c-terminus towards the membrane, this situation is different in FAK. In fact, an N-terminal FAK- YN-label, may indeed escape BiFC when FAK is in an active membrane bound conformation (via the FERM domain). On the other hand, an inactive form of FAK, present in the cytoplasm may allow a confirmation in which the N-terminal FERM domain is closer to the c-terminal FAT- domain, carrying the potential Y925-P residue targeted by PTP1B.

We only made YN/YC amino-terminal fusions of FAK because we did not want to perturb the focal adhesion targeting motif at the C-terminus. Previous reports have demonstrated that even short peptide tags added at the C-terminus of FAK can block the binding of Grb2, a known FAK interactor at this region (Cary et al, J Cell Biol 140: 211, 1998).

8. The inhibition of BiFC between PTP1B and paxillin by FRNK overexpression does not make sense to me. This FAK-fragment is recruited to focal adhesion bound paxillin that is likely to be normally phosphorylated, although the literature may propose otherwise. Please verify with anti-P- Pax antibodies whether in this specific context of FRNK expression the potential P-pax and respective PTP1B target is absent in these cells. Accordingly, if the paxillin BiFC is due to the recruitment of PTP1B to the P-Y925 of FAK, overexpression of FRNK might also affect the latter interaction in focal adhesions, but potentially not in central-locations in the cell.

New data. We know from previous studies that FRNK expression abrogates the Src/FAK-dependent phosphorylation of paxillin (González Wusener et al, Biol Open 5: 32, 2016, Fig. 6I-L) and also prevents the accumulation of the substrate trap mutant PTP1B DA in adhesions (Burdisso et al, J

Cell Sci 126: 1820, 2013, Fig. 6G and H), suggesting the absence of a target phosphoprotein under this condition. Our present BiFC results are consistent with those previous studies from our group and propose that two phosphoprotein substrates are paxillin and FAK. Thus, according to our results the basal BiFC between PTP1B DA and paxillin in the presence of FRNK is likely due to the displacement of active FAK from adhesions and to the reduced phosphorylation of paxillin.

As mentioned above, cells co-expressing FRNK and in FAK KO cells it is expected that paxillin phosphorylation is severely reduced as reported (Richardson and Parsons, Nature 380: 538, 1996; Tilghman et al J Cell Sci 118: 2613, 2005; Lim et al J Cell Biol 180: 187, 2008). We have also included additional panels showing the reduction of anti-pY118 signal under these conditions in new Fig S6, and a short text in Results, lines 234 and 236.

9. Molecular dynamics simulation and helical structure of the Y925-containing peptide: Indeed molecular dynamics analysis can be performed in order to analyse the stability of the FAT domain, for example in response to Y-925 phosphorylation. Based on the potential recognition of this sequence by PTP1B it would even be very relevant to know why the phosphorylated FAT domain can not bind paxillin, while associating easily with PTP1B. Structural information of phosphorylated Tyrosine containing peptides, clearly show that such sequences adopt a beta-sheet structure, which is maintained when bound to an SH2 domain. Indeed, such a beta-sheet locally unwinding helix-1 would likely disrupt the FAT-domain helix bundle, especially when bound to adapter proteins. Thus, considering such additional scenarios, or that of the PTP1B target site, being able to accommodate a p-tyrosine sidechain within a helical conformation could be of high interest to better understand the functions of site-specific phosphatase activities. Maybe a word on the conformation of the active sites of PTP's would be helpful for the readers.

Our answer to this comment of the reviewer is split in two new paragraphs added to the edited manuscript: One written under the title: "FAK and paxillin interaction in PTP1B WT and KO cells" in Results, lines 334-338 as follows: *"Structural analysis has revealed that FAK binding to paxillin only occurs when the FAT domain has a compact, four-helix bundle conformation (Gao et al., 2004; Hayashi et al., 2002; Hoellerer et al., 2003; Liu et al., 2002). Further co-immunoprecipitation studies establish that in this compact conformation FAK Y925 is in the non-phosphorylated state (Deramaudt et al., 2011; Kadaré et al., 2015) and not accessible to tyrosine kinases (Arold, 2011).* The second paragraph is under the title "Phosphorylation state of Y925 affects the stability of the FAT 4-helix bundle" in Results, lines 353-362: *"NMR and molecular dynamic simulations suggest that structural dynamics of the FAT domain leads to an alternative "open" conformation in which the helix 1 separates from the rest of the bundle and acquires the conformational freedom required for phosphorylation Prutzman et al., 2004; Zhou et al., 2006), observations consistent with crystallographic studies (Arold et al., 2002). The open conformation of the FAT domain prevents the binding with paxillin (Kadaré et al., 2015) and the more extended conformation of helix 1 may satisfy the tyrosine kinase and Grb2 requirements for binding (Brown et al., 1999; Hubbard, 1997; Rahuel et al., 1996). Since similar peptide conformations are bound by PTP1B (Jia et al., 1995; Salmeen et al., 2000) we predict that PTP1B binds to the phosphorylated Y925 in the open conformation of the FAT domain.*

Minor issues:
You could indicate the non-transfected control cells in panel S1B for their endogenous anti-paxillin reactivity.

We pointed the transfected (T) and non transfected (NT) cells stained with anti-paxillin in the new Fig. S2.

Merging green with red should be changed to green and magenta (follow guidelines for color-bling readers)

We changed the merge panels to green/magenta.

Figure 1 western blots: The low expression/recognition of FAK-5F and 3F by the anti-FAK antibodies should be clarified. Since anti-GFP is a completely different antibody, its reactivity could be much stronger than that of anti-FAK. Therefore for the anti-GFP blot, at least one additional FAK construct that is normally recognized by the anti-FAK antibody should be included. If FAK- 5F and

3F are much less expressed than the other construct, it is normal that BiFC with PTP-1B is reduced.

We clarified the limitation of the anti-FAK antibody in the legend of the Fig. 1. “Anti-FAK does not efficiently recognize FAK mutants YN-5F and YN-3F, probably due to the protein fragment (amino acids 354-533) used as immunogen. Thus, for these mutants, cell extracts were also probed with anti-GFP, which efficiently recognizes the YN fragment.” We repeated this text fragment on the Results section. We are sure that FAK-3F and 5F express at similar levels of the other constructs, as shown in the representative panels of Fig. 1C. All FAK images were taken using identical acquisition settings.

The type of imaging; e.g. confocal, epifluorescence, TIRF, should also be indicated in the legend of each figure.

Done

Legend to figure S2: legend is not in accordance to panels C and G: the text states anti-GFP to BiFC ratio, but measured was anti-paxillin? Please clarify, and in case anti-GFP was used in combination with BiFC, the quantitative analysis might be meaningless, because you do not know the relationship between both transfected proteins.

The reviewer is correct, we made a mistake in the legend, the text should say “BiFC to anti-paxillin ratio”. We corrected this.

Second decision letter

MS ID#: JOCES/2021/258769

MS TITLE: PROTEIN TYROSINE PHOSPHATASE 1B, PTP1B, TARGETS FAK AND PAXILLIN IN CELL-MATRIX ADHESIONS

AUTHORS: Ana E González Wusener, Ángela González, María E. Perez Collado, Melina R. Maza, Ignacio J General, and Carlos O. Arregui
ARTICLE TYPE: Research Article

I have returned your revised manuscript to the original reviewers #1 and #3. As you will see, reviewer #1 finds that you have satisfactorily addressed his/her comments and recommends publication. Reviewer #3 agrees that the manuscript has been improved, but still has some minor issues that you may want to address before submitting your final manuscript. I trust that you will be able to properly deal with them and look forward to receiving a final revision of your paperWe 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.)

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

In this revised manuscript, the authors have done an excellent job of addressing my concerns. I have no other concerns and recommend publication.

Comments for the author

In this revised manuscript, the authors have done an excellent job of addressing my concerns. I have no other concerns and recommend publication of this interesting study.

Reviewer 3

Advance summary and potential significance to field

The manuscript has been highly improved and I would like to thank the authors for the extensive revisions performed. As the general message of the manuscript has improved, it forms now also an excellent example, how cell-based cross-linking techniques, coupled with substrate trapping mutants can visualize protein-enzyme interactions that are normally too fast and too dynamic to being observed with more classical co-localization techniques. It is really impressive how such a technique can be used to identify a specific targeting and dephosphorylation event of PTP1B in a living cell, occurring on either the paxillin P-Y31/P-Y118, or FAK P-Y925. Although the regulatory model proposed in Fig S8 can be discussed, the manuscript offers also additional insights and observations that could be analyzed in further studies. For example, the subtle changes in the localization of the paxillin/PTP1B and FAK/PTP1B BiFC pairs could be an excellent way to further characterize the cycling of different types of integrin adapters between different cellular compartments or differentially matured integrin-adhesion structures.

To make the manuscript even more compelling, especially regarding the consequences of the different phosphorylation events studied. I would strongly suggest to the authors to make a few minor changes to the text, and highlight information from the literature that would be relevant for the types of constructs used and the physiological changes in focal adhesions observed.

Comments for the author

1. When introducing the paxillin BiFC construct (N-terminal tagged; Line 159), it would be relevant to mention a recent study (Ripamonti etal. 2021, cited), which extensively used N- and C-terminal tagged BiFC probes of paxillin and which showed the specific interaction with b3-integrin, only with a C-terminal tagged paxillin construct, but not the N-terminal tagged. This study by Ripamonti et al, is in fact a good argument why N-terminal paxillin tagging should be used for this current study and that the specific localization of the BIFC tag is relevant for the successful formation of the YN/YC complex, when studying cytoplasmic regulators such as PTP1B or FAK.

2. Furthermore, in the discussion (Line 438), it is proposed that paxillin phosphorylation on Y31/Y118 is enhancing adhesion dynamics, potentially through FAK recruitment. This dynamic aspect is certainly relevant, but this phosphorylation event is also relevant for the structural stability of focal adhesions. Ripamonti et al, (2021) have indeed shown and measured that Y31 and Y118 phosphorylation enhances the lifetime of paxillin in focal adhesions by a factor of 2, which would have an important impact on the stability of focal adhesions and their ECM-binding capacity. Whether this phosphorylation event is actually force dependent is another highly interesting question, but not really relevant for this study (Line 439). However, while the authors concentrate on the potential role in paxillin binding to the FAT domain of FAK, the same paxillin

phosphorylation would also likely enhance interaction with structural proteins, such as talin and vinculin, thereby stabilizing the adhesion structure. In turn, the phosphorylation on Y925 of FAK could actually enhance dissociation from paxillin and subsequent interactions with talin and vinculin.

Obviously this discussion can be easily extended, but the information that paxillin phosphorylation on Y31 and Y118 enhances its stability in focal adhesion would be really important for understanding how PTP1B activity in focal adhesions regulates adhesion-site stability, and should therefore be mentioned here in a sentence.

3. According to the papers hypothesis, in addition to paxillin phosphorylation, controlling the phosphorylation of Y925 in FAK, would indeed be another way to fine-tune the adhesion structure by regulating either its stabilization or turnover (see discussion above). Figure 6 clearly shows an example where focal adhesion stability is enhanced by PTP1B expression. While wildtype cells show a compact focal adhesion structure and prominent pax/FAK-BiFC, the PTP1B-KO-cells show reduced FAK/Pax interaction, but also more elongated adhesion structures that could indicate a failure to properly stabilize focal adhesions (by either too much P-Y925, or not enough Src/P-Y31 and P-Y118, obviously a nice switch for adhesion regulation). Interestingly, such elongated adhesions, is a phenotype quite similar to the adhesion sliding phenotype observed by Ripamonti et al (2021). In that paper, adhesion sliding was induced due to an improper mechanical coupling of paxillin to integrin receptors, therefore suggesting that a failure of PTP1B activity is causing a defect in adhesion maturity. (If the author repeatedly observed such changes in the adhesion structure, or reported it before, this correlation could be mentioned in the text. However, an additional analysis of the adhesion phenotype may go beyond the scope of the current manuscript).

4. I do not get the logic of figure S6J: Why should this panel be “PTP1B WT” cells and not “FAK WT” as panels S6G-I are taken from “FAK KO” cells?

Second revision

Author response to reviewers' comments

PLEASE REFER TO SUPPL INFO TO SEE THE ANSWER TO THE REVIEWER IN THE ORIGINAL FORMAT.

Reviewer 3

1. When introducing the paxillin BiFC construct (N-terminal tagged; Line 159), it would be relevant to mention a recent study (Ripamonti etal. 2021, cited), which extensively used N- and C-terminal tagged BiFC probes of paxillin and which showed the specific interaction with b3-integrin, only with a C-terminal tagged paxillin construct, but not the N-terminal tagged. This study by Ripamonti et al, is in fact a good argument why N-terminal paxillin tagging should be used for this current study and that the specific localization of the BIFC tag is relevant for the successful formation of the YN/YC complex, when studying cytoplasmic regulators such as PTP1B or FAK.

AUTHOR: We agree with the reviewer, and the following sentence was added between lines 159-161 of the Results section. “The N-terminus tagging of paxillin did not affect the interactions of the C-terminus LIM domains with focal adhesion proteins and with the plasma membrane (Ripamonti et al., 2021).”

2.Furthermore, in the discussion (Line 438), it is proposed that paxillin phosphorylation on Y31/Y118 is enhancing adhesion dynamics, potentially through FAK recruitment. This dynamic aspect is certainly relevant, but this phosphorylation event is also relevant for the structural stability of focal adhesions. Ripamonti et al, (2021) have indeed shown and measured that Y31 and Y118 phosphorylation enhances the lifetime of paxillin in focal adhesions by a factor of 2, which would have an important impact on the stability of focal adhesions and their ECM-binding capacity. Whether this phosphorylation event is actually force dependent is another highly interesting question, but not really relevant for this study (Line 439). However, while the authors concentrate

on the potential role in paxillin binding to the FAT domain of FAK, the same paxillin phosphorylation would also likely enhance interaction with structural proteins, such as talin and vinculin, thereby stabilizing the adhesion structure. In turn, the phosphorylation on Y925 of FAK could actually enhance dissociation from paxillin and subsequent interactions with talin and vinculin.

Obviously this discussion can be easily extended, but the information that paxillin phosphorylation on Y31 and Y118 enhances its stability in focal adhesion would be really important for understanding how PTP1B activity in focal adhesions regulates adhesion-site stability, and should therefore be mentioned here in a sentence.
AUTHOR: Following the reviewer's suggestion we have now added a new sentence between lines 439-442 and 474-477 at the Discussion section.

3.According to the papers hypothesis, in addition to paxillin phosphorylation, controlling the phosphorylation of Y925 in FAK, would indeed be another way to fine-tune the adhesion structure by regulating either its stabilization or turnover (see discussion above). Figure 6 clearly shows an example where focal adhesion stability is enhanced by PTP1B expression. While wildtype cells show a compact focal adhesion structure and prominent pax/FAK-BiFC, the PTP1B-KO-cells show reduced FAK/Pax interaction, but also more elongated adhesion structures that could indicate a failure to properly stabilize focal adhesions (by either too much P-Y925, or not enough Src/P-Y31 and P-Y118, obviously a nice switch for adhesion regulation). Interestingly, such elongated adhesions, is a phenotype quite similar to the adhesion sliding phenotype observed by Ripamonti et al (2021). In that paper, adhesion sliding was induced due to an improper mechanical coupling of paxillin to integrin receptors, therefore suggesting that a failure of PTP1B activity is causing a defect in adhesion maturity. (If the author repeatedly observed such changes in the adhesion structure, or reported it before, this correlation could be mentioned in the text. However, an additional analysis of the adhesion phenotype may go beyond the scope of the current manuscript).

AUTHOR: We agree with the reviewer that by keeping the FAK Y925 in the unphosphorylated state, PTP1B may promote the structural stability of focal complexes. On these lines we added a new paragraph between lines 525-527 on the Discussion section.

4. I do not get the logic of figure S6J: Why should this panel be "PTP1B WT" cells and not "FAK WT" as panels S6G-I are taken from "FAK KO" cells?

AUTHOR: Supplementary Fig. S6G-I was added to show that in the absence of FAK expression the levels of paxillin phosphorylation is reduced to background levels, a condition that explains the lack of BiFC between the substrate trap PTP1B DA and paxillin in FAK KO cells (Fig. 3K,L,M). Panel "J" was added for comparison with a cell expressing FAK. In the original figure we put a PTP1B WT cell, which expresses FAK. But following the suggestion of the reviewer we replaced the original panel by a new one from a FAK WT cell. We corrected this in the legend.

Third decision letter

MS ID#: JOCES/2021/258769

MS TITLE: PROTEIN TYROSINE PHOSPHATASE 1B, PTP1B, TARGETS FAK AND PAXILLIN IN CELL-MATRIX ADHESIONS

AUTHORS: Ana E González Wusener, Ángela González, María E. Perez Collado, Melina R. Maza, Ignacio J General, and Carlos O. Arregui
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.