Membrane stretching activates calcium-permeability of a putative channel Pkd2 during fission yeast cytokinesis

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(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.)

RE: Manuscript #E22-07-0248
TITLE: Membrane stretching activates calcium-permeability of a putative channel Pkd2 during fission yeast cytokinesis

Dear Dr Chen,

Thanks for sending your work to MBoC. Your paper has now been assessed by two experts in the field. You will see that both reviewers find your work interesting but also raise a number of important questions. Reviewer 1 asks in particular for an important negative control for the cell free expression system, while reviewer 2 expresses concerns on the rate of the calcium flux and use of the Gadolinium inhibitor.

While unable to accept your manuscript at this point, I will be happy to receive a revised version if you are able to address these and the other comments from the reviewers.

Please also make sure you follow the MBoC submission checklist, to ensure that you have indicated precise sample size and methodology.

Yours sincerely,

Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chen,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

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To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The manuscript by Liu and Chen groups investigated the mechanism of polycystin Pkd2 functions in fission yeast cell growth and division. Previous works from the Chen lab have found that Pkd2 localizes to intracellular vesicles and the plasma membrane and is essential in fission yeast cytokinesis. His lab has also pioneered a biosensor for calcium spike in S. pombe. In the current collaborated study, they combined elegant in vitro channel reconstitution and quantitative in vivo cell/genetic experiments to show Pkd2 is indeed a calcium channel. First, the authors expressed Pkd2-sfGFP in human HeLa cells based cell free expression system. Then they reconstituted Pkd2 as a transmembrane protein in supported lipid bilayers with extra reservoir (SUPER) templates. The in vitro system can permeate calcium under osmotic pressure and membrane stretching. In a series of high quality in vivo experiments using fission yeast mutants and microscopy, they found that Pkd2 is important for cellular calcium levels and calcium spikes in both dividing and non-dividing cells.

The findings in this study are novel and interesting. The data and figures are clearly presented and of high quality. I do not have major concerns on the impressive manuscript. I would like the authors to address or clarify my following concerns/questions.
1. I think a negative control is needed for the cell free expression (CFE) system: express sfGFP alone or non-channel transmembrane protein-sfGFP to be used in parallel experiments. To be honest, I have not used the CFE system. It's a very cool and efficient way to express a membrane protein. Without the negative control, how are you sure Pkd2 is responsible for the calcium permeation? Is it possible that some human proteins (like human polycystin or other proteins) in the CFE system functions as or help PkD2 to serve as the calcium channels? If you think the control is not necessary, please explain why.
2. In Fig. 1B, how did you detect Pkd2-sfGFP and the protein ladder? Was Pkd2 detected by coomassie staining or GFP signal? Was there Pkd2 without sfGFP in the CFE system? Was Pkd2-sfGFP much more abundant than human proteins when added to the SUPER templates? I noticed that Pkd2-sfGFP has a 6His tag. Any enrichment or purification using the tag?
3. I did not figure out why green lysine was used in the experiments presented in Fig. S2. How specific is green lysine? Why didn't you use Pkd2-sfGFP?
4. In figures, if n < 100 cells, it is better to give the exact numbers instead of n > 32 cells etc.
5. Although the Poddar et al., 2021 MBoC paper is cited, it's better to briefly explain how GCaMP-mCherry works as a calcium sensor in Materials and Methods. Because cell volumes change during osmotic shock, and pkd2 mutants deflate and reflate quickly, can line scan along cell long axis and normalization using mCherry account for the volume changes? Is it more accurate to use total GCaMP intensity to measure calcium levels in the whole cell?
6. Because cells of ura4+ and ura4- have very different growth rates, it's important to list the complete genotype in the strain table, such as ura4, ade6, leu1, his, lys1 etc, especially the ura4 genotype.

Reviewer #2 (Remarks to the Author):

Pkd2 is the fission yeast homolog of polycystins. The authors correctly state that the channel activity of Pkd2 in intact cells is not established. This paper addressed the issue of Pkd2 as a channel in fission yeast and suggests that Pkd2 allows calcium influx when activated by membrane stretching. These results suggest that Pkd2 is mechanosensitive, is needed for calcium flux, and provides a function, a contribution to the calcium spikes associated with cytokinesis. These findings have the potential to be an important addition to the work on polycystins, but there are several clarifications required before the story is complete.

The authors assert that Pkd2 localizes to the plasma membrane. That appears correct in their system, but they ignore the protein associated with the endoplasmic reticulum (ER). Work from their own lab (Morris et al, 2019) and prior work (Protchenko, 2006) in yeast show Pkd2 in the ER, as is expected from mammalian studies. In fact, the majority of Pkd2 is in the ER, not the plasma membrane or the cilia. The tone of the paper seems to provide surprise that there is any contribution from ER localized Pkd2. They end the paper with the assertion that Pkd2 is primarily in the ciliary membrane (line 296), but that is incorrect. The lack of acknowledgement that Pkd2 is highly expressed in the ER and could provide an important contribution in that location needs to be included. All discrepancies need to be corrected.

In all the figures with the reconstituted Pks2 in vesicles, the measured rate of calcium flux is very slow. With even one channel per vesicle, even in vesicles of the size used in this study, the rate of calcium flux into the vesicle should be saturated quickly, probably in less than a second. The authors suggest that the lack of calcium buffering inside the vesicles would slow flux, but that should lead to saturation faster. Please explain this discrepancy.

Line 105 and Fig 1B: Is the expressed protein (plus GFP) the correct molecular weight? Mammalian Pkd2 is 100KDa, plus GFP is 25-30KDa, so the total should be larger than what is shown in Fig 1B

Lines 124-126: Most prior assessments of the membrane location of Pkd2 show both C and N termini in the cytoplasm, and only 6 transmembrane helices. What is the rational for using only Alpha-fold, rather than experimentally determined orientations and cryo-EM structural studies?

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Line 252: The authors suggest that other cations could permeate. Pkd2 has been shown to be permeable to many cations (DeCaen, Delling, Kleene among others) including large organic cations (Anyatonwu, JBC 2005). Please include this info.

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The findings in this study are novel and interesting. The data and figures are clearly presented and of high quality. I do not have major concerns on the impressive manuscript. I would like the authors to address or clarify my following concerns/questions.

Response: We thank the reviewer for careful reading of our manuscript and for supporting its publication. We have now addressed all the concerned raised by the reviewer, as detailed below in a point-by-point rebuttal. The changes have also been highlighted in the manuscript for the reviewer's convenience.

1. I think a negative control is needed for the cell free expression (CFE) system: express sfGFP alone or non-channel transmembrane protein-sfGFP to be used in parallel experiments. To be honest, I have not used the CFE system. It's a very cool and efficient way to express a membrane protein. Without the negative control, how are you sure Pkd2 is responsible for the calcium permeation? Is it possible that some human proteins (like human polycystin or other proteins) in the CFE system functions as or help PkD2 to serve as the calcium channels? If you think the control is not necessary, please explain why.

Response: We thank the reviewer for raising this important point. A negative control without the expression of Pkd2 is indeed essential for us to draw such a conclusion. It has been presented in Fig. 2C (top row), in which GUV without Pkd2 did not allow any calcium influx, regardless of the osmotic condition. In this experiment, the G-GECO encapsulated within GUVs was expressed using the same HeLa-based CFE system used to express Pkd2. This negative result thus ruled out the possibility that some human proteins in the CFE system might participate in the forced-activated calcium influx. To highlight this point in the revised manuscript, we added (Line 153-155) *"In comparison, the fluorescence changed very litter under the similar condition in those GUVs without Pkd2 (Fig. 2C)."*.

2. In Fig. 1B, how did you detect Pkd2-sfGFP and the protein ladder? Was Pkd2 detected by coomassie staining or GFP signal? Was there Pkd2 without sfGFP in the CFE system? **Was Pkd2-sfGFP much more abundant than human proteins when added to the SUPER templates?** I noticed that Pkd2-sfGFP has a 6His tag. Any enrichment or purification using the tag?

Response: In the experiment described in Fig. 1B, Pkd2 was tagged with sfGFP and the protein ladder was fluorescently labeled with DyLight 650. We imaged the gel using a fluorescence gel scanner, not by coomassie staining.

In this experiment, we only expressed Pkd2 fused to sfGFP, so there shall be no untagged Pkd2 in the CFE system. We are not sure whether Pkd2-sfGFP was more abundant than human proteins on SUPER templates. Neither do we know which human proteins becomes incorporated in the SUPER templates. Nevertheless, due to the strong promoter of the expression vector, we assumed that Pkd2-sfGFP was much more abundant than the endogenous human polycystins in our CFE system.

Although Pkd2-sfGFP was also tagged with His_6 tag from the CFE expression vector, we did not utilize this tag for any purification or enrichment of Pkd2.

3. I did not figure out why green lysine was used in the experiments presented in Fig. S2. How specific is green lysine? Why didn't you use Pkd2-sfGFP?

Response: We used green lysine for fluorescent labeling and detection of in vitro synthesized Pkd2 in the place of GFP in the above-mentioned experiment. It is based on a lysine-charged tRNA labeled with the fluorophore BODIPY®-FL at the epsilon amino group. It can incorporate into synthesized proteins during in vitro translation reactions and has been used by many groups. Since there are no other translation reactions happening in the reaction other than the intended expression, this approach specifically labeled untagged proteins.

We agreed with the reviewer that this experiment can equally be done with Pkd2-sfGFP (in some ways similar to Figure 1), but we wanted to test the wild-type untagged protein. Therefore, we used green lysine to track Pkd2.

4. In figures, if n < 100 cells, it is better to give the exact numbers instead of n > 32 cells etc.

Response: We made the changes suggested by the reviewer. Wherever there are <100 cells, we have added the exact number of cells analyzed in figures (Fig. 4 and 5).

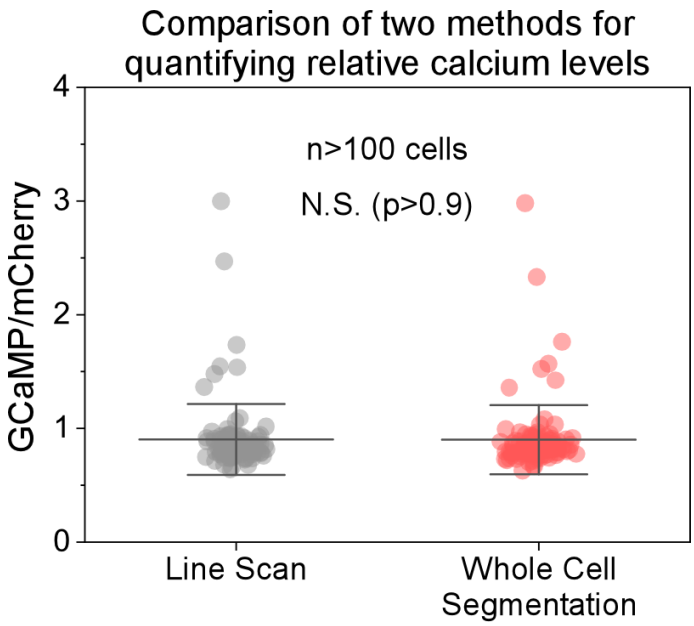
5. Although the Poddar et al., 2021 MBoC paper is cited, it's better to briefly + and pkd2 mutants deflate and reflate quickly, can line scan along cell long axis and normalization using mCherry account for the volume changes? Is it more accurate to use total GCaMP intensity to measure calcium levels in the whole cell?

Response: We thank the reviewer for raising this important point in regard to the choice of fluorescence calcium indicator. We chose GCaMP-mCherry over GCaMP to measure the equilibrated calcium level of the cells without stimulation, (Fig 3) because only the former could be used for the ratio-metric measurement to account for the intracellular concentration of the fluorescence indicator. The concentration of calcium indictors was much higher in the *pkd2* mutant cells, due to their cell size expansion defect, compared to the wild type (Sinha et al., 2022). By measuring the ratio of GCaMP to mCherry fluorescence, we accounted for this difference in cell growth between the wild type and the mutant.

To clarify this method, we have added the following clarification in the Materials and Methods section (Line 513-516) *"GCaMP to mCherry fluorescence ratio gave a more accurate measurement than GCaMP alone for quantifying the calcium levels because it took into consideration the intracellular concentration of the calcium reporter."*

The reviewer had a good point about the deflation defect. We did take the temporary deflation of the *pkd2* mutant cells (Morris et al., 2019; Sinha et al., 2022) into consideration. We measured the calcium level of both deflated and non-deflated cells separately. However, we did not find a significant difference between them ($p>0.05$). Therefore, the data presented in Fig. 3A, and B included both deflated and non-deflated cells. To clarify, we added the following in the revised text (Line 181-182) *"However, the calcium levels of deflated and non-deflated pkd2-81KD cells were not statistically different"*.

The reviewer was correct that it would have been more straightforward to measure the average intracellular GCaMP fluorescence by segmenting the individual cells, rather than using the line-scan method. However, the manual measurement of single cells by the line-scan method is far less tedious, and it produced almost identical measurements, compared to the whole-cell segmentation (Fig. R1). This is the primary reason that we chose the line-scan method in our study.



Rebuttal Figure R1: Comparison of the line-scan and whole-cell segmentation methods of measuring intracellular calcium level in the wild type fission yeast cells. A scatter plot of the GCaMP to mCherry fluorescence ratio measured by these two methods separately. N.S.: no significant difference (two-tailed student t-tests). Data pooled from two independent biological repeats.

6. Because cells of ura4+ and ura4- have very different growth rates, it's important to list the complete genotype in the strain table, such as ura4, ade6, leu1, his, lys1, etc., especially the ura4 genotype.

Response: Thanks for the reviewer pointing this out! We have added the auxotrophic genotype of all the yeast strains in the revised table S1.

Reviewer #2 (Remarks to the Author):

Pkd2 is the fission yeast homolog of polycystins. The authors correctly state that the channel activity of Pkd2 in intact cells is not established. This paper addressed the issue of Pkd2 as a channel in fission yeast and suggests that Pkd2 allows calcium influx when activated by membrane stretching. These results suggest that Pkd2 is mechanosensitive, is needed for calcium flux, and provides a function, a contribution to the calcium spikes associated with cytokinesis. These findings have the potential to be an important addition to the work on polycystins, but there are several clarifications required before the story is complete.

Response: We thank the reviewer for carefully reviewing our manuscript and for providing constructive criticism. We have now addressed all the concerns raised by the reviewer, most importantly in regard to the intracellular localization of fission yeast Pkd2. A point-by-point rebuttal is below. The changes have also been highlighted in the manuscript for the reviewer's convenience.

The authors assert that Pkd2 localizes to the plasma membrane. That appears correct in their system, but they ignore the protein associated with the endoplasmic reticulum (ER). Work from their own lab (Morris et al, 2019) and prior work (Protchenko, 2006) in yeast show Pkd2 in the ER, as is expected from mammalian studies. In fact, the majority of Pkd2 is in the ER, not the plasma membrane or the cilia. The tone of the paper seems to provide surprise that there is any contribution from ER-localized Pkd2. They end the paper with the assertion that Pkd2 is primarily in the ciliary membrane (line 296), but that is incorrect. The lack of acknowledgment that Pkd2 is highly expressed in the ER and could provide an important contribution in that location needs to be included. All discrepancies need to be corrected.

Response: We thank the reviewer for raising the critical point of the intracellular localization of Pkd2. Our previous studies have demonstrated that fission yeast Pkd2 mostly localizes to both the plasma membrane and intracellular membrane structures (Morris et al., 2019). However, we never determined whether a fraction of Pkd2 proteins may localize to the ER. To address this question directly, we constructed a new strain expressing both the endogenously tagged Pkd2-GFP and the ER marker mCherry-ADEL and imaged it with confocal fluorescence microscopy.

Our new data strongly supported our previous results that Pkd2 primarily localizes to the plasma membrane instead of the ER (Fig. S5A and replicated below). First, Pkd2-GFP localized to the equatorial plane during cytokinesis (asterisk), while the ER marker mCherry-ADEL did not. Secondly, consistent with other studies (Vjestica et al., 2020; Zhang et al., 2012), the ER distributed prominently both at the perinuclear region (arrowhead) and in the membrane tethers between the cortical and perinuclear ERs (arrow). In contrast, Pkd2 was missing from both locations. Nevertheless, we cannot completely rule out the possibility that a small portion of Pkd2 may localize at the cortical ER, which is separated by a space of mere ~50nm from the plasma membrane in fission yeast (Cortes et al., 2012; Martin-Cuadrado et al., 2005; Wang et al., 2002). We address this possibility in the revised discussion (line 285-287) *"Since the yeast cortical ER is adjoined to the plasma membrane, it is possible that a very small fraction of Pkd2 may localize to the ER like the human polycystin PC-2"*

This primary plasma membrane localization of fission yeast Pkd2 clearly differs from that of human PC-2. However, we would like to point out that there is only one polycystin homologue in fission yeast. Therefore, Pkd2 may represent the ancestor of both PC-2 and PC-1. The latter indeed primarily localizes to the plasma membrane in animal cells (Ibraghimov-Beskrovnaya et al., 1997).

We thank the reviewer for correcting our original statement regarding the localization of human PC-2. We revised it as the following (line 163-172) *"Since the human polycystin PC-2 is found in the ER (Cai et al., 1999), we first determined whether Pkd2 also localizes to the fission yeast endoplasmic reticulum (ER). We examined the cells expressing both Pkd2-GFP and the ER marker, mCherry-ADEL with fluorescence microscopy (Fig. S5A). Although both fluorescence proteins localized to the cell periphery, this is most likely due to that Pkd2 localized to the plasma membrane (Morris et al., 2019) which is separated by a distance of less than 100 nm from the yeast cortical ER (Zhang et al., 2010). In*

contrary, Pkd2-GFP localized to the equatorial plane during cytokinesis, while the ER marker did not. Moreover, Pkd2 was absent from either the perinuclear ER or the ER tethers, both of which were marked by mCherry-ADEL (Fig. S5A). We concluded that the calcium-permeable Pkd2 primarily localizes to the plasma membrane"

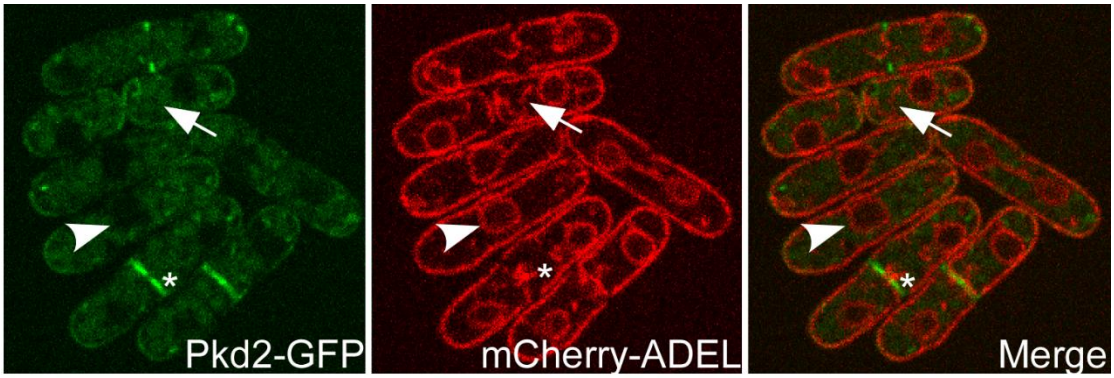


Figure S5A: Pkd2 primarily localizes to the plasma membrane. Fluorescence micrograph of the cells expressing both Pkd2-GFP (green) and the ER marker mCherry-ADEL (red). Only the center slice of the Z-series is shown. Asterisk: Equatorial plane localization, Arrowhead: Perinuclear ER, Arrow: Membrane tethers.

In all the figures with the reconstituted Pkd2 in vesicles, the measured rate of calcium flux is very slow. With even one channel per vesicle, even in vesicles of the size used in this study, the rate of calcium flux into the vesicle should be saturated quickly, probably in less than a second. The authors suggest that the lack of calcium buffering inside the vesicles would slow flux, but that should lead to saturation faster. Please explain this discrepancy.

Response: Thanks for raising this important point in our in vitro study! Actually, the force-activated calcium influx can be detected in 1 minute. However, the fluorescence of G-GECO is visible but very dim at the beginning. It did take some time to reach saturation. This might be due to the small number of reconstituted channels in the membrane as compared to the amount of endogenous Pkd2 in the yeast cells. This may slow the calcium influx. The in vitro experiments were used to establish a relationship between membrane stretching (by osmotic shock) and calcium permeation. We have refrained from drawing any quantitative conclusions on rate of calcium influx as this requires more detailed knowledge on the number of Pkd2 channels and G-GECO expression level that is at present difficult to determine precisely.

We thank the reviewer for pointing out the seemingly backward logic of ours on the lack of calcium buffering contributing to slow calcium flux. We have removed the sentence.

Line 105 and Fig 1B: Is the expressed protein (plus GFP) the correct molecular weight? Mammalian Pkd2 is 100KDa, plus GFP is 25-30KDa, so the total should be larger than what is shown in Fig 1B

Response: The molecular weight of fission yeast Pkd2 is 80 KD, not 100KD. Therefore, the molecular weight of Pkd2-sfGFP, detected in the fluorescence gel, is 105 KD. We added the following clarification (Line 106-109) *"The expressed protein appeared as a single band of ~105 kDa on an SDS-PAGE gel (Fig. 1B), which was consistent with the predicted molecular weight of the fusion protein, MW_{Pkd2}=80 kDa and MW_{GFP}=27 kDa"*.

Lines 124-126: Most prior assessments of the membrane location of Pkd2 show both C and N termini in the cytoplasm, and only 6 transmembrane helices. What is the rationale for using only Alpha-fold, rather than experimentally determined orientations and cryo-EM structural studies?

Response: Unlike the human polycystins, the structure of Pkd2 has not been experimentally determined. Its putative structure predicted by AlphaFold, differs significantly from that of mammalian polycystin-2. It possesses 9, instead of 6 transmembrane helices, with an extracellular N-terminus and a cytoplasmic C-terminus, which was described in one of our recent preprint (Malla et al., 2022). We have added the following clarification (line 128-129). "*Unlike the human polycystins, the structure of fission yeast Pkd2 has not been experimentally determined".*

Line 150: Another Pkd2 inhibitor that is not a multi-charged cation needs to be included. Gadolinium could have many other effects, including displacing calcium and altering the membrane structure, especially in the presence of negatively charged lipids. The non-specificity of gadolinium and the slowness of the flux could imply that calcium is slowly leaking into the vesicles by a non-channel pathway.

Response: We thank the reviewer for pointing to the potential side effects of gadolinium. Unfortunately, we are not aware of other Pkd2 inhibitors in its place. Nevertheless, our in vitro study has provided other controls to make sure that Pkd2 is responsible for the calcium influx into the GUVs in vitro. First, the GUV without Pkd2 did not exhibit leakage of calcium (Fig. 2C). Although we only show the images taken 10 minutes after applying osmotic shock here, we did not observe any leakage of calcium based on the G-GECO fluorescence for over 1 hour. Secondly, the force-activated calcium influx can be detected as fast as in 1 minute and the fluorescence of G-GECO inside GUVs increased within 5 minutes (Fig. 2E).

Line 252: The authors suggest that other cations could permeate. Pkd2 has been shown to be permeable to many cations (DeCaen, Delling, Kleene among others) including large organic cations (Anyatonwu, JBC 2005). Please include this info.

Response: Thanks for pointing this out! We have added the recommend references as the following (line 278-280) *"It remains to be determined whether Fission yeast Pkd2 is also permeable to other cations such as potassium, sodium, and organic cations similar to human polycystins (Anyatonwu and Ehrlich, 2005; Ha et al., 2020; Kleene and Kleene, 2017; Liu et al., 2018)".*

Minor

Line 63: "in vitro" missing a space

Response: We have corrected this mistake.

Line 259 and other places: What does "it" refer to? Language needs some clarification

Response: We revised this sentence for clarification by replacing it with Pkd2. Now it reads (Line 288) *"Consistent with the mechanosensitivity in vitro, Pkd2 also plays a critical role in adaptation to hypo-osmotic shock when tension of the plasma membrane increases".* We have clarified such references in other places in the revised text.

Line 260: "as we have shown before", fix grammar

Response: We found this wording at line 216, not at 260, and corrected it.

Line 265: should be "spikes"

Response: We have corrected this mistake.

Line 288: should be "in contrast"

Response: We have corrected this mistake.

Rebuttal Letter References:

Cortes, J.C., M. Sato, J. Munoz, M.B. Moreno, J.A. Clemente-Ramos, M. Ramos, H. Okada, M. Osumi, A. Duran, and J.C. Ribas. 2012. Fission yeast Ags1 confers the essential septum strength needed for safe gradual cell abscission. *The Journal of cell biology*. 198:637-656.

Ibraghimov-Beskrovnaya, O., W.R. Dackowski, L. Foggensteiner, N. Coleman, S. Thiru, L.R. Petry, T.C. Burn, T.D. Connors, T. Van Raay, J. Bradley, F. Qian, L.F. Onuchic, T.J. Watnick, K. Piontek, R.M. Hakim, G.M. Landes, G.G. Germino, R. Sandford, and K.W. Klinger. 1997. Polycystin: in vitro synthesis, in vivo tissue expression, and subcellular localization identifies a large membrane-associated protein. *Proceedings of the National Academy of Sciences of the United States of America*. 94:6397-6402.

Malla, M., D. Sinha, P. Chowdhury, B.T. Bisesi, and Q. Chen. 2022. Cytoplasmic tail of the putative polycystin channel Pkd2 regulates its clustering in the fission yeast eisosomes. *bioRxiv*.

Martin-Cuadrado, A.B., J.L. Morrell, M. Konomi, H. An, C. Petit, M. Osumi, M. Balasubramanian, K.L. Gould, F. Del Rey, and C.R. de Aldana. 2005. Role of septins and the exocyst complex in the function of hydrolytic enzymes responsible for fission yeast cell separation. *Molecular biology of the cell*. 16:4867-4881.

Morris, Z., D. Sinha, A. Poddar, B. Morris, and Q. Chen. 2019. Fission yeast TRP channel Pkd2p localizes to the cleavage furrow and regulates cell separation during cytokinesis. *Molecular biology of the cell*. 30:1791-1804.

Sinha, D., D. Ivan, E. Gibbs, M. Chetluru, J. Goss, and Q. Chen. 2022. Fission yeast polycystin Pkd2p promotes cell size expansion and antagonizes the Hippo-related SIN pathway. *Journal of cell science*. 135.

Vjestica, A., M. Marek, P.J. Nkosi, L. Merlini, G. Liu, M. Berard, I. Billault-Chaumartin, and S.G. Martin. 2020. A toolbox of stable integration vectors in the fission yeast Schizosaccharomyces pombe. *J Cell Sci*. 133.

Wang, H., X. Tang, J. Liu, S. Trautmann, D. Balasundaram, D. McCollum, and M.K. Balasubramanian. 2002. The multiprotein exocyst complex is essential for cell separation in Schizosaccharomyces pombe. *Molecular biology of the cell*. 13:515-529.

Zhang, D., A. Vjestica, and S. Oliferenko. 2012. Plasma membrane tethering of the cortical ER necessitates its finely reticulated architecture. *Curr Biol*. 22:2048-2052.

RE: Manuscript #E22-07-0248R
TITLE: "Membrane stretching activates calcium-permeability of a putative channel Pkd2 during fission yeast cytokinesis"

Dear Dr. Chen:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. This makes a very nice contribution to our understanding of cytokinesis.

Sincerely,
Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chen:

Congratulations on the acceptance of your manuscript.

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