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Low kindlin-3 levels in osteoclasts of kindlin-3 hypomorphic mice result in osteopetrosis due to leaky sealing zones

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Original submission

First decision letter

MS ID#: JOCES/2021/259040

MS TITLE: Low kindlin-3 levels in osteoclasts of kindlin-3 hypomorphic mice result in osteopetrosis due to leaky sealing zones

AUTHORS: Sarah Klapproth, Karsten Richter, Clara Tuerk, Theresa Bock, Thomas Bromberger, Julian Dominik, Kathrin Huck, Kristian Pfaller, Michael W. Hess, Christoph A. Reichel, Marcus Krueger, Inaam A. Nakchbandi, and Markus Moser
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

Overall, this article is interesting and develops new ideas that will be of interest to the readership of JCS.
However, there are a number of areas that need significant improvement before this manuscript is ready for publication.

1) There is not enough background information regarding Kindlin families in bone environment. Since this article focuses on Kindlin-3, it is necessary for the readers to have a general background about Kindlin family members and their diverse functions in regulating bone development and homeostasis. As far as I know, there are increasing evidences that show the importance of Kindlin-2 participating in skeletal development, bone homeostasis and bone mechanotransduction.

2) Since bone mass maintenance is a balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption, it would be important to include some data that show the changes of osteoblast formation and function in the control and Kindlin-3 hypomorphic mice.

3) For the generation of Kindlin-3 hypomorphic mice, it is suggested to include detailed description of this mouse strain at the first paragraph of Results section.

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2 in these cells.

5) Authors published the involvement of Kindlin-3 in osteoclast integrin activation and podosome formation during bone homeostasis in 2011 (Schmidt et al., 2011), it is suggested to have a detailed discussion to empathize the major findings of current study.

6) Some minor errors for the wiring of genotype, such as K3n/-, and gene and protein names, such as Kindlin-3.

Comments for the author

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Reviewer 2

Advance summary and potential significance to field

In this paper, Klapproth et al., provide insights into the mechanism by which low kindlin-3 levels (5-10% expression) affects osteoclast function leading to mild osteopetrosis in mice. They utilize in vitro techniques to show reduced kndlin3 levels in osteoclasts results in defective cell spreading, adhesion, signaling, abnormal podosomes, and most importantly impaired sealing zones. In vivo using kindlin3 hypomorphic mouse model, they demonstrate increased BMD in long bones indicative of mild osteoporosis.

Finally, they utilize electron microscopy to impressively demonstrate impaired sealing zones and ruffled border of osteoclasts in kindlin3 hypomorphic mice. The overall phenomenon has been described by the same group, making this manuscript somewhat confirmative. However, several aspects of the underlying mechanism are new and very interesting and therefore, deserve an in-depth analysis. The manuscript definitely fits the Journal profile.

Comments for the author

In this paper, Klapproth et al., provide insights into the mechanism by which low kindlin-3 levels (5-10% expression) affect osteoclast function leading to mild osteopetrosis in mice. The paper is logical and all conclusions are well supported. The overall methodological approaches are similar to those utilized previously by the same group showing that osteopetrosis in Kindlin 3 deficient mice due to impaired osteoclast function. However, several aspects of the underlying mechanism are new and very interesting and, therefore, deserve an in-depth analysis.
The suggestion is to enhance the mechanistic aspects of the story.

1. Fig 1C: could the delay in osteoclast differentiation by kindlin3 hypomorph bone marrow cells be due to increased apoptosis? Reduced kindlin3 might affect bone marrow cells and osteoclast cell survival. This seems to be an important point to address.
2. Figure 1E: There is a need to confirm the generation of pre-osteoclasts in all genotypes before western analysis. Kindlin3 deficiency might delay pre-osteoclast generation as 2.5 days was used for all genotypes including WT.
3. It is very crucial that localization of Kindlin3 in +/+ cells is shown. Does it in fact localize to the podosomes, podosome belt, and sealing zones?
4. Colocalization of kindlin3 with phospho-paxillin to membrane/podosome belt in +/+ and its absence in hypomorph cells should be shown.
5. Fig2: Localization of phospho-paxillin rather than total paxillin is more informative as it is the phospho-paxillin levels that change with reduced kindling3 levels as shown in fig1E.
6. Fig 2G: It is unclear how low kindlin3 levels directly affect actin cytoskeleton organization in the adhesion belt?
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8. -/- phenotype is shown randomly in some experiments but absent in others. Either add -/- to all or remove.
9. In vivo kindlin3 hypomorph mice show increased BMD. Do osteoblasts express kindlin3? Since these are global kindlin3 hypomorph could osteoblasts play a role in increased BMD along with dysfunctional osteoclasts?
10. Fig 4f: why are there a higher number of osteoclasts in kindling hypos? If the kindlin3 hypos are unable to adhere and migrate, it is expected their number will below as this is a crucial step for osteoclast precursors.
11. Fig 4H, I: Why was p7 used for EM? 4.5 or 6-month mice should be shown as increased BMD was shown at these ages only. Also, the osteoclasts look abnormal in general, are they apoptotic and hence abnormal sealing zones and RB? This should be confirmed.
12. Fig 4D: n/- should be included.

Reviewer 3

Advance summary and potential significance to field

This study shows a new function of kindlin-3 as an organizer of an integrin42-mediated adhesion structure the sealing zone of the osteoclast.

Comments for the author

"Low kindlin-3 levels in osteoclasts 1 of kindlin-3 hypomorphic mice result in osteopetrosis due to leaky sealing zones", by Klapproth et al., used kindlin-3 hypomorphic mice to study osteoclast-like cell adhesion and function and effect on bone turnover in vivo.

In brief, low kindlin-3 expression reduced integrin activity, impaired osteoclast-like cell adhesion and signaling, and delayed cell spreading. In vitro kindlin-3-hypomorphic osteoclasts arrange their podosomes in adhesion belts but podosome and actin organization iwere abnormal, but cells were able to form sealing zones when on calcified matrix in vitro and on bone surface in vivo nevertheless. Functional assays and electron micrographs showed partial defects in resorption lacunae, causing mild osteopetrosis.

The assays are in general well written and the figures of high quality and easy to understand. I have only minor comments, enumerated below.

• Bone marrow monocytes with MCSF and RANKL make osteoclast-like cells. Not osteoclasts. The cells spread much more than real osteoclasts on bone and typically have distribution of acid phosphatase not all on the apical surface, typically little or no ruffled border, and many more nuclei than osteoclasts in vivo.
This does not affect the meaning of the work, but it is recommended that it be stated accurately. E.g.,
kindlin-3 hypomorphic osteoclast-like cells in Fig 1.

• Please do not refer to antibody labeling as "staining". The reviewer realizes that he is old fashioned but protein degrading enzymes are proteinases. Split infinitives should be removed, such as "fail to properly seal" in the abstract.

• Figure 2 is in general beautiful, but in Fig 2G it is not clear what is shown. If possible, please include a low power showing what cellular region is shown.

• In Figure 3, it is important to stress osteoclast-like since activity on mineralized matrix is less (practically none at all) than in vivo in Fig 4. Please specify in more detail what are "osteologic slides" (plates?) if memory serves plates coated with gelatin and mineral, not bone.

• Figure 4 is very nice.

First revision

Author response to reviewers' comments

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Reviewer 1 Comments for the Author:

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Response: We agree with the reviewer that the reader would benefit from more information on kindlin expression and the role of Kindlin-2 in bone and added it to the introduction.

2) Since bone mass maintenance is a balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption, it would be important to include some data that show the changes of osteoblast formation and function in the control and Kindlin-3 hypomorphic mice.

Response: This is indeed an important point. We thoroughly investigated osteoblasts from kindlin-3 deficient mice in a previous publication (Schmidt et al., 2011). Osteoblasts express kindlin-2, while kindlin-3 is not detectable in these cells. Osteoblast numbers per bone surface was normal in kindlin-3 knockout mice. Calvaria-derived osteoblasts from control and knockout mice were comparable in osteoblastic marker gene expression, alkaline phosphatase activity and mineralized bone nodule formation. Due to these results we decided not to study osteoblasts of hypomorphic mice, but list this important information in the manuscript.

3) For the generation of Kindlin-3 hypomorphic mice, it is suggested to include detailed description of this mouse strain at the first paragraph of Results section.

Response: We added more information about the kindlin-3 hypomorphic mouse to the manuscript. The main phenotypes are mentioned in the introduction and at the beginning of the results section. A detailed description of kindlin-3 hypomorphic mice can be found in our previous publication (Klapproth et al., 2015).

4) In Figure S1, authors compared the relative protein expression levels of Kindlin-3, Talin, Integrin-β1, Integrin-β2, Integrin-β3 in wild type macrophages and osteoclasts compared to platelets, neutrophils and fibroblasts. It is intriguing to know the expression of the other two Kindlin members, i.e., Kindlin-1 and Kindlin-2 in these cells.

Response: This question is often asked when it comes to the expression of kindlins in blood cells. All our previous studies revealed that hematopoietic cells exclusively express kindlin-3 and we never detected kindlin-3 in non-hematopoietic cells.
In our mass spectrometry experiments, Kindlin-1 was not detected in any of the protein lysates. Negligible amounts of kindlin-2 were detected in osteoclasts, macrophages, platelets and PMNs:

Table 1 for the reviewer: Relative abundance of kindlin-2 compared to kindlin-3

	Ratio normalized to the median of K3
Osteoclasts	0.008
Macrophages	0.002
Platelets	0.0003
Neutrophils	0.03

5) Authors published the involvement of Kindlin-3 in osteoclast integrin activation and podosome formation during bone homeostasis in 2011 (Schmidt et al., 2011), it is suggested to have a detailed discussion to empathize the major findings of current study.

Response: We thank the reviewer for the suggestion and tried to emphasize the major findings of the two studies in our discussion section.

6) Some minor errors for the wiring of genotype, such as K3n/-, and gene and protein names, such as Kindlin-3.

Response: We unified the naming of genotypes and corrected gene names.

Reviewer 2 Advance Summary and Potential Significance to Field:

In this paper, Klapproth et al., provide insights into the mechanism by which low kindlin-3 levels (5-10% expression) affects osteoclast function leading to mild osteopetrosis in mice. They utilize in vitro techniques to show reduced kndlin3 levels in osteoclasts results in defective cell spreading, adhesion, signaling, abnormal podosomes, and most importantly, impaired sealing zones. In vivo using kindlin3 hypomorphic mouse model, they demonstrate increased BMD in long bones indicative of mild osteoporosis. Finally, they utilize electron microscopy to impressively demonstrate impaired sealing zones and ruffled border of osteoclasts in kindlin3 hypomorphic mice. The overall phenomenon has been described by the same group, making this manuscript somewhat confirmative. However, several aspects of the underlying mechanism are new and very interesting and, therefore, deserve an in-depth analysis. The manuscript definitely fits the Journal profile.

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In this paper, Klapproth et al., provide insights into the mechanism by which low kindlin-3 levels (5-10% expression) affect osteoclast function leading to mild osteopetrosis in mice. The paper is logical and all conclusions are well supported. The overall methodological approaches are similar to those utilized previously by the same group showing that osteopetrosis in Kindlin 3 deficient mice due to impaired osteoclast function. However, several aspects of the underlying mechanism are new and very interesting and, therefore, deserve an in-depth analysis. The suggestion is to enhance the mechanistic aspects of the story.

1. Fig 1C: could the delay in osteoclast differentiation by kindlin3 hypomorph bone marrow cells be due to increased apoptosis? Reduced kindlin3 might affect bone marrow cells and osteoclast cell survival. This seems to be an important point to address.

Response: We thank the reviewer for this suggestion. To address this question, we performed TUNEL-assays on paraffin sections of bone and on *in vitro* differentiated (pre-)osteoclasts (Figure 1 for the reviewer A/B). We also prepared and analysed additional bone ultrasections by TEM but could not find any evidence of pyknotic or fragmented osteoclast nuclei (Figure 1 for the reviewer C).
We would like to clarify that reduced kindlin-3 expression does not result in delayed osteoclast differentiation, meaning fusion and osteoclast marker upregulation (see also answer to the next question), but in reorganization of the cytoskeleton. This can be rescued, at least in part, by exposure to high MCSF concentrations.

We would also like to note that all of our previous studies on our kindlin-3 knockout mouse model have never shown increased apoptosis in any of the investigated cell types studied (which seems to be different to kindlin-2 deficiency in chondrocytes and osteoblasts). We actually measured a reduced proliferation in thymocytes, as their interaction with antigen-presenting cells is integrin dependent (Moretti et al; 2018).

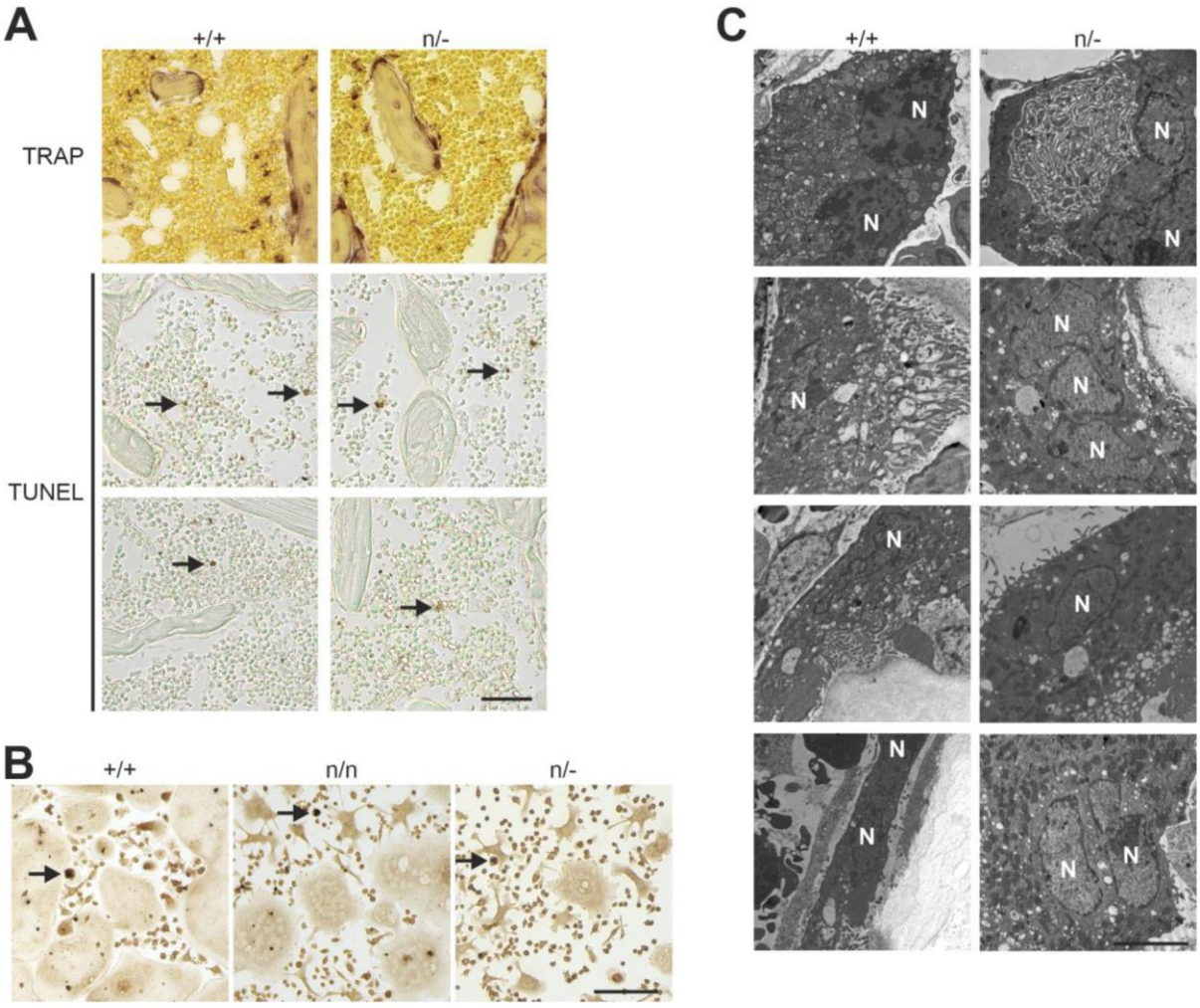

Figure 1 for the reviewer: Kindlin-3 hypomorphic osteoclasts do not show signs of increased apoptosis. (A) Staining for TRAP activity marks osteoclasts in histological paraffin sections of decalcified bone, TUNEL staining identifies apoptotic cells. Arrows point to TUNEL-positive, apoptotic bone marrow cells. Size bar 200 µm. (B) A TUNEL staining performed after 4 d of osteoclast differentiation revealed only few apoptotic cells (marked by an arrow). Size bar 200 µm. (C) Osteoclast nuclei imaged by electron microscopy on bone ultrasections of +/+ and n/- mice reveal no signs of apoptosis. Size bar 5 µm. N: Nucleus.

2. Figure 1E: There is a need to confirm the generation of pre-osteoclasts in all genotypes before western analysis. Kindlin3 deficiency might delay pre-osteoclast generation as 2.5 days was used for all genotypes including WT.

Response: We have previously analysed osteoclast differentiation by induction of osteoclast marker gene expression in the absence of kindlin-3 and found no major difference between control and kindlin-3 deficient cells (Schmidt et al., 2011). This indicates that kindlin-3 has no major impact on osteoclast differentiation. This important information is mentioned in line 127f. Additionally, we do not observe a delay in osteoclast fusion but only in spreading and cytoskeleton reorganization.

3. It is very crucial that localization of Kindlin3 in +/+ cells is shown. Does it in fact localize to the podosomes, podosome belt, and sealing zones?

Response: Localization of kindlin-3 to the podosome ring was already shown before (Ussar et al., 2006; Schmidt et al., 2011; Klapproth et al., 2019). Localization of kindlin-3 in podosome belts and sealing zones is now shown in new Figure S3.

4. Colocalization of kindlin3 with phospho-paxillin to membrane/podosome belt in +/+ and its absence in hypomorph cells should be shown.

Response: The integrin-mediated adhesion signaling assay shown in Figure 1E indeed suggests that low kindlin-3 expression results in reduced phospho-paxillin levels within the adhesion side. To investigate integrin-mediated adhesion signaling, starved cells were plated on osteopontin-coated plates for only 20 min, which allowed the cells to adhere and to initiate adhesion structure formation and signaling. Due to the reduced number of active integrins, integrin signaling indicated by Pyk2 and paxillin phosphorylation is reduced in kindlin3 hypomorphic cells (Fig 1E). Additionally, we have also shown previously that less kindlin-3 hypomorphic cells form podosomal structures compared to wild-type cells (Klapproth et al., 2015), which may further enhance the reduced paxillin phosphorylation levels in the whole cell lysates analysed in our western blot analysis. Figure 2A for the reviewer shows phospho-paxillin localisation to podosome rings within a podosome belt of a wildtype cell.
However, as shown in Figure 2B for the reviewer, phospho-paxillin levels are much higher in podosome clusters and podosome belts of cultured kindlin-3 hypomorphic pre-osteoclasts as shown by immunofluorescence labeling. We could recently decipher the molecular mechanism that leads to the increased phospho-paxillin levels within podosomes at low kindlin-3 expression (Klapproth et al., 2019). In this study, we could show that kindlin-3 recruits leupaxin to the adhesion complex, which in turn controls PTP-PEST enzymatic activity and reduces paxillin phosphorylation. Consequently, at low kindlin-3 levels, little leupaxin localizes to podosomes resulting in low activity of the phosphatase PTP-PEST and high paxillin phosphorylation (Klapproth et al., 2019). Thus, we believe that phospho-paxillin levels cannot accumulate during the short adhesion signaling experiment in contrast to cells that were cultured on glass to investigate the adhesion structures by immunofluorescene microscopy. Additionally, less cells form podosomal structures compared to wild-type cells (Klapproth et al., 2015).
These observations are rather counterintuitive and difficult to understand at a first sight. As we think that the manuscript does not benefit from this information, we would like to avoid showing this data, which is basically already published (Klapproth et al., 2019).

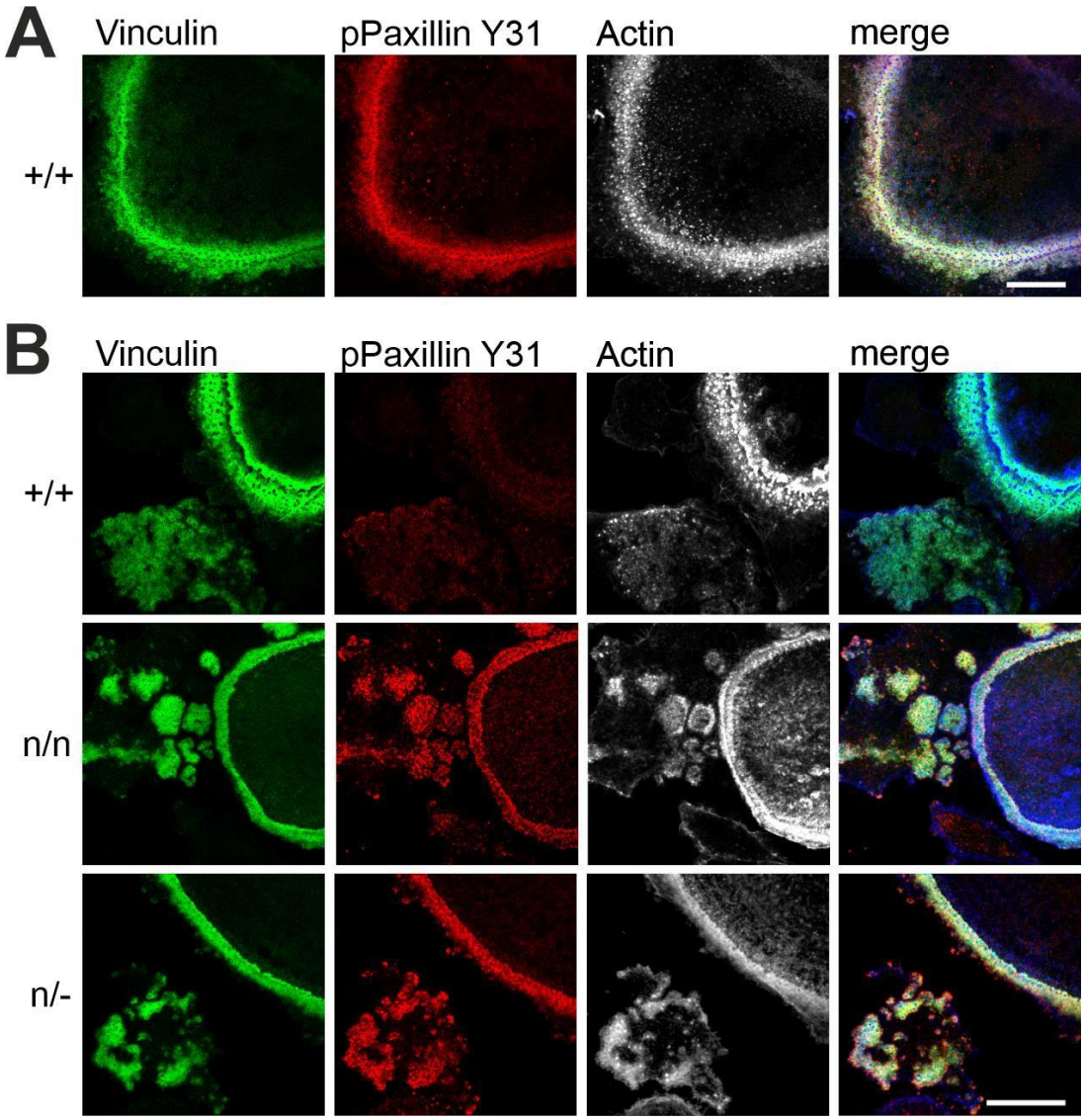

Figure 2 for the reviewer: Phospho-paxillin levels are high in adhesion structures of hypomorphic (pre)osteoclasts. (A) Confocal images of a wild-type osteoclast differentiated on glass coverslips and immunofluorescently labelled for vinculin, (green), phospho-paxillin Y31 (red) and actin (white, in merge blue) to show colocalization with vinculin. (B) Confocal images of wild-type and kindlin- 3 hypomorphic osteoclasts to visualize vinculin, (green), phospho-paxillin Y31 (red) and actin (white, in merge blue) localization. Exposure time for phospho-paxillin decreased compared to (A). Size bars 20 µm.

5. Fig2: Localization of phospho-paxillin rather than total paxillin is more informative as it is the phospho-paxillin levels that change with reduced kindling3 levels as shown in fig1E.

Response: We think that showing phospho-paxillin instead of total paxillin would be rather confusing to the reader due to the reasons explained above.

6. Fig 2G: It is unclear how low kindlin3 levels directly affect actin cytoskeleton organization in the adhesion belt?

Response: To our knowledge the molecular details how kindlin-3 regulates the maturation of the podosome actin core and their strong interconnection by stress fibers has not been investigated in detail. Possible mechanisms of how kindlin-3 regulates and organizes the actin cytoskeleton through kindlin-3 interactors and kindin-3-dependent signaling pathways are now listed in the Discussion.

7. Line 207 and Fig 3B and C: why were fetal liver cells used instead of bone marrow cells that were used for all the previous experiments? It is suggested to keep the cell type consistent.

Response: Kindlin-3 knockout mice die early after birth. Due to the early lethality and to the severe osteopetrotic phenotype we were not able to isolate sufficient numbers of bone marrow cells for osteoclast differentiation. This information is now given in the text.

8.-/- phenotype is shown randomly in some experiments but absent in others. Either add -/- to all or remove.

Response: We show data from kindlin-3 knockout cells to demonstrate the degree of the defect in hypomorphic cells compared to full knockout cells. This is to emphasize that even with appr. 5% kindlin-3 some integrin-dependent activity (quantified for adhesion, signaling, resorption) is detectable. In other words, the knockout cells serve here as the "null"-standard for quantitative measurements. However, since K3-deficient osteoclasts completely fail to form podosome belts or sealing zones (Schmidt et al., 2011), we did not include this genotype for these studies. We would like to keep it like this, but are willing to remove these data in case it is confusing.

9. In vivo kindlin3 hypomorph mice show increased BMD. Do osteoblasts express kindlin3? Since these are global kindlin3 hypomorph could osteoblasts play a role in increased BMD along with dysfunctional osteoclasts?

Response: Please see answer to reviewer 1, question 2.

10. Fig 4f: why are there a higher number of osteoclasts in kindling hypos? If the kindlin3 hypos are unable to adhere and migrate, it is expected their number will below as this is a crucial step for osteoclast precursors.

Response: Kindlin-3 knockout mice, like kindlin-3 hypomorphic mice, also show strongly increased osteoclast numbers, and we have analysed the cause for this surprising finding in detail in our previous publication (Schmidt et al., 2011). Both lines show a strongly reduced ability to resorb bone matrix. Therefore, serum Ca^{2+} levels are strongly reduced, which is sensed to trigger the release of PTH from the parathyroid gland. PTH acts on cells of the osteoblast lineage, which then secrete RANKL that eventually induces osteoclast differentiation. Apparently, osteoclast differentiation *in vivo* is less dependent on adhesion and migration at high RANKL levels, especially when the density of precursors is high. We hope we have now made this point clearer in the discussion.

11. Fig 4H, I: Why was p7 used for EM? 4.5 or 6-month mice should be shown as increased BMD was shown at these ages only. Also, the osteoclasts look abnormal in general, are they apoptotic and hence abnormal sealing zones and RB? This should be confirmed.

Response: Showing bones from 1-week old mice is mainly for technical reasons, as cutting of old and, in our case, osteopetrotic bones is even more difficult. Importantly, the mechanism of bone resorption by osteoclasts does not change over time. In addition, especially during the initial growth of the animal, there is a lot of remodeling of the bone matrix, which increases the likelihood of finding active resorbing osteoclasts by TEM.

We did not find any signs of increased apoptotic activity in hypomorphic cells, neither in vivo nor in vitro (see also answer to question 1).

12. Fig 4D: n/- should be included.

Response: We aged a number of mice for 2 years. Unfortunately, most of the n/+ and the n/- mice died before or were used for other studies. Thus, only a sufficient number of control and n/n mice remained for this analysis.

Reviewer 3 Advance Summary and Potential Significance to Field:

This study shows a new function of kindlin-3 as an organizer of an integrin42-mediated adhesion structure, the sealing zone of the osteoclast.

Reviewer 3 Comments for the Author:

"Low kindlin-3 levels in osteoclasts 1 of kindlin-3 hypomorphic mice result in osteopetrosis due to leaky sealing zones", by Klapproth et al., used kindlin-3 hypomorphic mice to study osteoclast-like cell adhesion and function and effect on bone turnover in vivo. In brief, low kindlin-3 expression reduced integrin activity, impaired osteoclast-like cell adhesion and signaling, and delayed cell spreading. In vitro kindlin-3-hypomorphic osteoclasts arrange their podosomes in adhesion belts but podosome and actin organization iwere abnormal, but cells were able to form sealing zones when on calcified matrix in vitro and on bone surface in vivo nevertheless. Functional assays and electron micrographs showed partial defects in resorption lacunae, causing mild osteopetrosis. The assays are in general well written and the figures of high quality and easy to understand. I have only minor comments, enumerated below.

- Bone marrow monocytes with MCSF and RANKL make osteoclast-like cells. Not osteoclasts. The cells spread much more than real osteoclasts on bone and typically have distribution of acid phosphatase not all on the apical surface, typically little or no ruffled border, and many more nuclei than osteoclasts in vivo. This does not affect the meaning of the work, but it is recommended that it be stated accurately. E.g., kindlin-3 hypomorphic osteoclast-like cells in Fig 1.

Response: Thanks for this clarification - we corrected this.

- Please do not refer to antibody labeling as "staining". The reviewer realizes that he is old fashioned but protein degrading enzymes are proteinases. Split infinitives should be removed, such as "fail to properly seal" in the abstract.

Response: Thank you very much! We corrected these points in the text and hope we learned that lesson!

- Figure 2 is in general beautiful, but in Fig 2G it is not clear what is shown. If possible, please include a low power showing what cellular region is shown.

Response: The reviewer is right. We therefore prepared a new Figure S4, including a low power magnification. Arrows now mark the cell border.

- In Figure 3, it is important to stress osteoclast-like since activity on mineralized matrix is less (practically none at all) than in vivo in Fig 4. Please specify in more detail what are "osteologic slides" (plates?) if memory serves plates coated with gelatin and mineral, not bone.

Response: Biocoat^{TM} Osteologic^{TM} slides were purchased from BD Biosciences. These are coverslips of 12.7 mm diameter coated with proprietary bone biomaterial. Detailed information about the composition of this material is not available. Unfortunately, these very useful slides are not available anymore.

- Figure 4 is very nice.

Thank you very much!

Second decision letter

MS ID#: JOCES/2021/259040

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ARTICLE TYPE: Research Article

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

Reviewer 1

Advance summary and potential significance to field

The authors have answered all the questions asked and updated the manuscript. This revised version is ready for publication.

Comments for the author

No further questions.

Reviewer 2

Advance summary and potential significance to field

This paper provides insights into the mechanism by which low kindlin-3 levels affects osteoclast function leading to mild osteopetrosis in mice. The utilization of in vitro studies supported by in vivo analysis including electron microscopy to demonstrate impaired sealing zones and ruffled border of osteoclasts in kindlin3 hypomorphic mice is impressive. The manuscript shows several aspects of the underlying mechanism that are very interesting. This manuscript definitely fits the Journal profile.

Comments for the author

The authors have address all my concerns to satisfaction. The revised version is comprehensive and well documented.
Only one minor concern: Include stats for Fig 1B.