JCB

Repair of nuclear ruptures requires barrier-to-autointegration factor

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Corresponding Author(s): Kyle Roux, Sanford Research/USD

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February 16, 2019

Re: JCB manuscript #201901116

Dr. Kyle J Roux
Sanford Research/USD
2301 N 60th St. East
Sioux Falls, SD 57104

Dear Dr. Roux,

Thank you for submitting your manuscript entitled "Repair of nuclear rupture requires barrier-to-autointegration factor". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

The reviewers -- and we agree -- found the results interesting and exciting. They however shared some suggestions to ensure the data are technically rigorous and support the conclusions. Rev#1 suggested a number of changes that can be largely addressed by text and figure edits, and we find this expert's suggestions constructive and helpful to clarify the results and interpretation. Please address all of Rev#1's points -- which in our view would not require more experimentation, including point #4.

You will see that our other two reviewers, #2 and #3, shared more substantial concerns. Rev#2 stressed that the work does not have the level of mechanistic depth expected from a JCB Article. To this referee, key mechanistic questions revolve around the role of BAF phosphorylation and whether BAF-induced repair involves LEM domain membrane proteins that bind BAF as the inner nuclear membrane diffuses to fill the gap or occurs via cytoplasmic vesicles containing newly synthesized nuclear membrane proteins. Ref#2 additionally felt that you did not rule out the possibility that BAF would initially go against a gradient of other proteins exiting the nucleus that would slow its initial moving further beyond the rupture site; the referee would recommend FRET analyses to track proteins appearing in the cytoplasm after rupture to test if their movement could be affecting BAF dynamics/entry.

Rev#3 asked for more quantitative analyses (#1), particularly the dynamics of BAF mutants. Additionally, the ref felt that you should at least discuss the involvement of the ESCRT machinery (point #2), given the known role of BAF in post-mitotic nuclear envelope reassembly and the known and important role of ESCRT complexes in NE repair, and they suggested testing whether all LEM proteins are important or redundant in NE repair (#3).

We discussed these points in depth editorially. We did consider at submission the somewhat limited extent of the mechanistic analyses and share Rev#2's concerns. We agree with the referee that the work provides novel and interesting observations, which we agree with Rev#2 fit the JCB Report format quite nicely. JCB Reports must provide definitive findings of high interest to a broad audience with the potential to open up new avenues of research. Thus, with adequate revisions to strengthen the observations, we agree with the referee that the work would be very competitive in the Report format. We therefore suggest that you reformat the work and submit the revision as a

Report. Reports can have up to 5 main and 3 supplemental figures, so the work already fits the limits and each figure can span up to one entire page. In our view, there is room in the manuscript to address the reviewers' points. The character count (see below) is limited to 20,000, but please note that we are flexible on this count, within reason. We're happy to discuss this change further as needed.

Please address Rev#2's points by careful additions to the manuscript. No experimentation is needed at this stage to tackle these major mechanistic questions, which would require a significant amount of work, but these points should certainly be discussed. On the other hand, we suggest that you focus experimental efforts in revision on tackling Rev#3's points. We find these points from this expert in the field valid and important. The quantifications are needed to make the claims convincing and this is in line with standards for the journal. We also agree with the referee that the omission of the ESCRT question is relatively glaring and Rev#3 suggested clever but seemingly straightforward experimental additions that would deepen the impact of the work and help better place your results and hypothesis about BAF function in repair in the context of the known existing mechanisms of repair, so we encourage you to follow through on the very nice suggestions from this referee.

Please let us know if you would like to discuss the revisions further or anticipate any issues addressing the reviewers' points. We would be happy to discuss these points further.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

Our typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments

point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to the Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Michael Rout, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

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

This manuscript is a beautifully mechanistic study that shows a direct role for barrier to autointegration factor in repairing sites of nuclear envelope rupture. They studied cells in which nuclei were ruptured by mechanical pressure or (in most cases) using a laser. They confirmed the previously-reported rapid localization of BAF at sites of rupture, and then demonstrated co-enrichment of the LEM-domain protein LEMD2 (Fig 1). They showed that GFP-BAF concentrates in the nucleus after rupture, and by photobleaching either the cytosolic or nucleosolic populations of GFP-BAF showed that the cytoplasmic pool is predominantly recruited to rupture sites (Fig 2). They expressed BAF mutations that disrupt binding to DNA (K6A) or LEM-domains (L58R), or both, or a triple-mutant (EEE) that mimics phosphorylation near the BAF N-terminus [which residues?] and found that localization at sites of rupture was slowed most dramatically by reduced binding to DNA (Fig 3). BAF is required to recruit three GFP-fused LEM-domain proteins (LEMD2, Man1, Emerin) to rupture sites (Fig 4, Fig 5), demonstrating a requirement for BAF and revealing the recruitment of LEM-domain proteins as a proposed second step in this repair pathway. The timecourse of repair is addressed in Figure 5. Overall this is a rigorous and well-written study that reveals a fascinating mechanism by which nuclear ruptures are repaired.

The manuscript requires revision to address the questions below.

1. The gene encoding BAF (BANF1) must be named in the Abstract/p2 and Introduction/p3 to avoid confusion with other proteins known as BAFs (Brahma Associated Factors).

2. The K6A mutation used to reduce binding to DNA, also reduces/abolishes binding to histones H3 and H1 (Montes de Oca et al., 2005, JBC). This is relevant, and should be considered in the Discussion (e.g., Discussion page 7).

3. The specific BAF residues affected by the phospho-mimetic MEEEQ construct must be identified on page 5; they are Thr2, Thr3 and Ser4. A nearby phospho-mimetic mutation (K6E) that abolishes binding to DNA, also reduces binding to emerin (Segura-Totten et al., 2002, JCB).

4. Briefly discuss (rule out?) whether the BAF mutations used in this study, might also affect binding

to lamins A/C (interface includes BAF residues Ala12, Pro14, Phe88, maybe also Asp86; Samson et al., 2018, NAR).

5. Figure 3B is confusing, unnecessary, and detracts from the manuscript. Overexpressing VRK1 caused an artificial increase in the amount of phosphorylated BAF in the cytoplasm, as detected by a phospho-BAF-specific antibody, but this increased cytoplasmic BAF was not detected by a 'total BAF' antibody. I agree with the authors that native BAF is notoriously difficult to detect via antibodies; this makes Figure 3B inconclusive, so what is the point?

6. Page 6, line 3: clarify which data supports the statement "enrichment [of GFP-tagged LEM domain proteins] was persistent for tens of minutes (Figure 1D)" -- Figure 1D shows the 5 minute timepoint only, for a single LEM protein (LEMD2).

7. Page 6, "Similar to GFP-LEM protein recruitment, depletion of BAF... when monitored for up to 15 minutes". Where is 15-minute data shown? (Fig 4 only shows 5-min).

8. Page 6, bottom paragraph, a more accurate interpretation of Figure 5 may allow the authors to deduce a timeline for plugging versus repair. E.g.: "but by 5-10 minutes the reporter clearly began to shift back into the nucleus, likely coincident with repair of the rupture (Figure 5A-B, Movie S8)." This interpretation is inaccurate. mCherry-NLS signals in the nucleus look similar at 1-15 min in Fig 5A, suggesting the situation is stable; Fig 5A does not show re-accumulation in the nucleus. Similarly, the 'flatlined' cytoplasmic/nucleoplasmic ratio for the NLS marker with overlapping error bars in Fig 5B suggests that net leakage from the nucleus stops (ie., rupture plugged) within ~6 minutes in wildtype cells. (Fig 5B does not address re-accumulation in the nucleus). When cells lack BAF, leakage appears to continue until ~10-12 minutes.
A longer timeline with constriction-generate ruptures is shown in Fig 5C: control cells appear to rapidly 'plug' the leak, with evidence for partial re-accumulation at the 30 min timepoint (15 min after rupture), and restored nuclear accumulation by ~2 hours. The si-BAF cells are still 'leaking' at 60 minutes (45 min after rupture), and then flatline (achieve 'plug'?) by ~90 minutes, but show no evidence of restored nuclear import.
Revise Discussion accordingly.

9. Page 6 ("To test how loss of BAF affects nuclear rupture..."): Fig 5C addresses the timecourse of repair, not "rupture". Suggest deleting "Yet unlike with the loss of lamin a/C, the loss of" and changing this final sentence to "We conclude that BAF is uniquely required to repair the NE after nuclear rupture."

10. Page 7, paragraph 3, "BAF has no substantial intercompartmental mobility" is not true for S-phase: BAF is predominantly nuclear during S-phase, suggesting potent cell cycle regulation of intercompartmental mobility (Haraguchi et al., 2007 JCS).

11. Page 7, last paragraph, lines 1-2 do not do justice to your model that BAF binds exposed DNA/chromatin, and then rapidly recruits LEM-domain proteins with their associated membranes. Agree that other proteins may also participate (lamins, and/or other INM proteins). There is direct evidence for substantial 'free' populations of emerin at the NE, based on solubility and non-association with lamins/chromatin (Berk et al, 2013 JBC; Holaska & Wilson, 2007).

12. Results and Legend for Figure 3C: Which differences were statistically significant?

13. Figure 3C is uninterpretable in black and white. Use larger and distinct shapes (e.g., circles,

triangles, squares, open or black) for each construct. Consider moving neighboring error bars slightly to right or left, so they can be distinguished.

14. Figure 5D (model): Depicting the NE as a single line is unacceptable in the context of this biology. Depict a true NE (inner membrane, outer membrane, thin lumen) in the schematic, and true ER in the bottom panel. The bottom panel should also show that BAF might recruit LEM-proteins in adjacent NE (current panel implies ALL rescuing membrane is from cytoplasm).

Minor corrections or improvements:

Page 3, paragraph 1: Delete "Within" and in remainder of sentence replace "that is most proximate to the nuclear contents reside a host of transmembrane" with "has many resident transmembrane".

Page 3, paragraph 3: "BAF binds to dsDNA as a monomer" is misleading, because BAF forms obligate dimers. A corrected statement, e.g.: "BAF forms obligate dimers, each subunit of which binds dsDNA allowing BAF to 'bridge' two strands of dsDNA".

Page 6: Include the measured impact on mechanical force resistance, e.g., "We found that ~20% less force was needed to rupture the nucleus in cells downregulated for either BAF or A-type lamins (Figure S3)." This result is consistent with evidence that BAF strengthens lamin A/C binding to LEM-domain proteins (Samson et al., 2018).

Page 7, Discussion paragraph 4, line 3: also cite Samwer et al (2017).

Page 8: Why is cholera toxin added to MCF10A culture medium?

Legend to Figure 2C: The images are representative of how many total cells examined?

Legend to Figure 4, line 3: Change "Man1, and Emerin" to "Man1 or Emerin". Line 5: change to "expressing each GFP-tagged... protein"

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

In "Repair of nuclear rupture requires barrier-to-autointegration factor" by Halfmann and colleagues the authors have presented a very novel hypothesis that after nuclear rupture cytoplasmic BAF enters through the hole and binds to exposed chromatin and since BAF can bind to LEM domain nuclear membrane proteins such as emerin it helps to recruit these proteins to the site of rupture to seal the break. This hypothesis is the more attractive since it makes sense with a number of existing data. The microscopy data shows clearly that GFP-BAF targets to the rupture site rapidly and that it can be observed to sequentially migrate further into the nucleus over time. This latter point in itself would be simple common sense, but the dynamics of the further migration as assessed by the live cell imaging would be further consistent with an initial function at the periphery in rupture sealing. Of note, I was particularly pleased that they used multiple approaches to induce rupture since the first approach with the laser-induced ruptures could have lots of secondary effects on this system and mechanism of repair because of local heating while the pillars bend membranes at non-physiological angles that cause leakage and other though different artefacts so that it is important to show these effects through multiple approaches for inducing rupture. At the same time, their data could also be consistent with a number of other interpretations such as

initially going against a gradient of other proteins exiting the nucleus that would slow its initial moving further beyond the rupture site and no experiments were done to test this alternate hypothesis such as following proteins at the same time shown in previous studies by other groups studying nuclear rupture and autophagy that show nuclear proteins that appear in the cytoplasm after rupture to determine if their migration out of the cell could be slowing the entry of the BAF up to a certain point. There also were no experiments testing whether BAF has a slightly protracted interaction with these LEM domain proteins or other chromatin proteins in the initial step. FRET could reasonably easily address this issue or FCCS for more depth. While the experiments using BAF mutations is extremely compelling support for the hypothesis, it was not investigated in much detail where doing the FRET or FCCS experiments with the simple microscopy would strengthen this by better clarifying the mechanism.

Another mechanistic question that was briefly alluded to by the authors was the potential role of BAF phosphorylation where they tested the effect of VRK1 overexpression, but there were several logical extensions of this experiment missing and outstanding mechanistic questions on this part. For example, what happens when you use a degron to deplete VRK1 after it is no longer required in mitosis? What happens if you block BAF and/or nuclear membrane protein phosphorylation? If a cyto-nuc prep was done to separate cytoplasmic and nuclear BAF, what is the phosphorylation distribution measured in this particular cell line and how effectively does each pool bind to naked DNA, nucleosomes, or nuclear membrane proteins? Is cytoplasmic BAF getting modified once it enters the nucleus and if it is not getting modified then why is it not getting exported once the rupture is sealed? While aspects of BAF nucleocytoplasmic transport remain elusive, the tendency towards further nucleoplasmic migration instead of nuclear export with this pool is interesting and these simple experiments can be done without knowing the details of its import/export pathways.

Probably the most core mechanistic question is does BAF-mediated membrane repair occur through LEM domain membrane proteins that bind BAF as the inner nuclear membrane diffuses to fill the gap or by cytoplasmic vesicles containing newly synthesized nuclear membrane proteins? This could be very simply addressed by separately activating cytoplasmic or nuclear envelope pools of the nuclear membrane protein with photoactivatable GFP shortly before the induced rupture as well as many other approaches including at least for a first approximation +/- cyclohexamide. The methods used to address the timing of membrane recruitment versus LEM domain protein did not yield a clearcut and strong result.

The paper itself is clearly written and I was much more pleased than when reviewing most papers with the discussion mostly qualifying the interpretation by limiting it to the results rather than overstating the meaning. In particular it clearly states the need for many of the types of experiments mentioned above before making clear conclusions. This is where my own confusion lies as I would have expected from the results shown for this to be submitted as a Short Report whereas it is listed as an Article. As I think the finding is novel and of import and the data shown are well controlled and ruptures induced by multiple approaches I would support this paper as a Short Report without further experimentation. However, for an Article there is more of an expectation for mechanistic insight and there is very little here and so I would recommend revision with addressing at least one of the two primary questions noted above in detail or both in less depth if it is to be published as an Article.

Reviewer #3 (Comments to the Authors (Required)):

Review of MS# 201901116, "Repair of nuclear rupture requires barrier-to-autointegration factor" by Halfmann et al.

Summary: Over the past few years there has been rapid progress in defining the molecular details that support repair of nuclear envelope (NE) ruptures in interphase, which appear to be highly related to the closure of the NE at mitotic exit. While previous work has hinted that BAF might be a molecular player in this pathway, this work by Halfmann et al. provides the first solid and thorough investigation of how BAF is functionally important in NE repair. The authors recapitulate previous findings that BAF is recruited to sites of NE rupture, which they introduce through several different mechanisms. They also take on the question of how BAF assembles at sites of NE rupture, providing intriguing evidence for a key role for nuclear compartmentalization of BAF phosphoregulation (in turn linked to DNA binding), with VRK phosphorylating BAF in the nucleus while cytoplasmic BAF is unmodified (and therefore primed to respond by binding to DNA at sites of NE rupture). The authors go on to demonstrate that BAF is essential for the recruitment of several LEM domain proteins to sites of nuclear rupture (namely emerin, LEMD2 (LEM2), and MAN1). Consistent with previous work suggesting that LEMD2/LEM2 is particularly critical for NE repair and/or closure of the NE at mitotic exit, the authors demonstrate that BAF depletion strongly inhibits reestablishment of the nuclear envelope barrier after rupture.

Assessment: Overall this is an interesting, timely, well-conceived and convincing study that provides new insights into the mechanisms governing NE repair. The data are of high quality and the paper is well-written and accessible. The authors are generally careful in their interpretations and, taken together, the observations make a nice story. As outlined below, my only criticisms focus on 1) the need to provide more quantitative analysis of some phenotypes, particularly the dynamics of BAF mutants; and 2) the somewhat glaring lack of discussion of the ESCRT machinery and other insights that have come from the study of NE closure at mitotic exit that are really critical to put this work into context. In addition, comparing the effects of BAF depletion with that of the established NE closure machinery (LEM2/CHMP7) would also provide deeper insight. While these are relatively minor issues, the effort to address them would improve the manuscript and its impact.

Specific Points:

1. The authors describe a clear difference in the recruitment of the DNA binding, LEM domain protein binding and phosphomimetic mutants of BAF compared to the WT. From the stills (Fig. 3A) and movies the described differences were not qualitatively obvious. The data may well support the authors' interpretation, but it would be very helpful to have some quantitative analysis to back up their statements (e.g. fluorescence intensity of BAF at the rupture, kinetics of accumulation and dissolution?). There is quantification of the nuclear exchange (Fig. 3C), but this comes later and it's not clear whether this reflects the affinity of the non-specific DNA binding and/or the off-rate from the accumulation at the rupture.
2. Given that the authors place BAF as critical to the recruitment of LEM domain proteins including LEM2/LEMD2, it seems very strange that they do not discuss the model built up over the past few years that LEM2 and its orthologues (from budding and fission yeasts) promote NE repair by recruiting the ESCRT-III machinery (Gu et al., PNAS, 2017; Webster et al., EMBO J, 2016). The role for ESCRTs also plays a prominent role in the Denais and Raab work. Without this piece the model seems incomplete, as one would expect that BAF is the far up-stream factor that ultimately engages the ESCRT machinery. Moreover, this work raises issues that really do warrant discussion in this manuscript (next point).
3. LEM2 was found to be critical for recruitment of the ESCRT machinery at mitotic exit (Gu et al). The authors' findings that BAF promotes recruitment of all tested LEM domain proteins raises the

question of whether there is specificity or redundancy. Given the very strong defect in reestablishing the NE barrier in BAF depleted cells (Fig. 5), it would be very helpful to know how depletion of only LEM2 or CHMP7 would quantitatively compare. Although this is a relatively small experiment, it would go a long way to shed light on whether all the LEM proteins are functionally relevant in the NE repair pathway, which remains an open and important question.

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SANFORD RESEARCH

April 29, 2019

The Journal of Cell Biology
The Rockefeller University Press
950 Third Ave., 2nd Floor
New York, NY 10022

Dear Editors,

We have prepared a revised manuscript #201901116 by Halfmann et. al. entitled “Repair of nuclear rupture requires recruitment of LEM-domain membrane proteins by barrier-to-autointegration factor” which we would like considered for publication as a Report in the *Journal of Cell Biology*.

We have tried to address as many of the critiques as possible from the reviews of the first submission.

The work described in our manuscript is laid out as follows: *Underlined text indicates new results in the revision.*
(1) Our results substantiate an earlier study that reported barrier-to-autointegration factor (BAF), a small DNA and LEM-domain binding protein that is essential for proliferative metazoan cellular life, localizes to sites of nuclear rupture. We have expanded upon this finding to demonstrate that BAF rapidly but transiently localizes to sites of nuclear rupture.

(2) It is predominantly a cytoplasmic population of non-phosphorylated BAF that localizes to nuclear rupture sites.

(3) DNA binding is a primary mechanism for BAF behavior in response to nuclear ruptures.

(4) Within a couple of minutes of nuclear rupture, following BAF recruitment, Emerin, Man1, LEMD2, Ankle2, Lap2α, and Chmp7 (ESCRT-III complex member) but not Lap2β are recruited to and enriched at sites of nuclear rupture in a BAF-dependent manner.

(5) BAF is required to recruit membranes to sites of nuclear rupture, and to functionally reseal the barrier formed by the nuclear envelope and its embedded nuclear pore complexes.

(6) LEMD2 is required to recruit Chmp7 to nuclear ruptures.

(7) Loss of LEMD2 or Chmp7 similarly do not prevent the repair of nuclear ruptures.

(8) Simultaneous depletion of LEMD2, Emerin and Ankle2 do prevent the repair of nuclear ruptures in a manner almost identical to the loss of BAF.

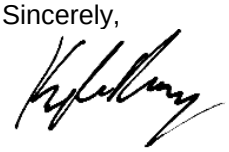
With the addition of new data and in order to keep the manuscript within the confines of the Report parameters as requested by the Editors our discussion is extremely limited and we have some removed discussion that was included in the original submission. This resulted in our inability to address all of the critiques and suggestions raised by reviewer #1 to improve the accuracy of the text, although we have tried to do so whenever possible. These substantial changes have also led us to not indicate where new text was added/changed for the sake of clarity. If this is not acceptable, we can mark these changes. We have added

a new figure (Figure 5) and combined data from figures in the first submission to keep the total figures within the limits of a Report.

It remains our opinion that this manuscript will appeal to a broad and growing audience of investigators studying the cell biology of nuclear rupture and its relationship to normal cell physiology and disease states.

Thank you for your consideration. The specific responses to reviewer comments follows.

Sincerely,



Kyle Roux, PhD
Scientist
Enabling Technologies Group
Sanford Research

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

This manuscript is a beautifully mechanistic study that shows a direct role for barrier to autointegration factor in repairing sites of nuclear envelope rupture. They studied cells in which nuclei were ruptured by mechanical pressure or (in most cases) using a laser. They confirmed the previously-reported rapid localization of BAF at sites of rupture, and then demonstrated co-enrichment of the LEM-domain protein LEMD2 (Fig 1). They showed that GFP-BAF concentrates in the nucleus after rupture, and by photobleaching either the cytosolic or nucleosolic populations of GFP-BAF showed that the cytoplasmic pool is predominantly recruited to rupture sites (Fig 2). They expressed BAF mutations that disrupt binding to DNA (K6A) or LEM-domains (L58R), or both, or a triple-mutant (EEE) that mimics phosphorylation near the BAF N-terminus [which residues?] and found that localization at sites of rupture was slowed most dramatically by reduced binding to DNA (Fig 3). BAF is required to recruit three GFP-fused LEM-domain proteins (LEMD2, Man1, Emerin) to rupture sites (Fig 4, Fig 5), demonstrating a requirement for BAF and revealing the recruitment of LEM-domain proteins as a proposed second step in this repair pathway. The timecourse of repair is addressed in Figure 5. Overall this is a rigorous and well-written study that reveals a fascinating mechanism by which nuclear ruptures are repaired.

The manuscript requires revision to address the questions below.

1. The gene encoding BAF (BANF1) must be named in the Abstract/p2 and Introduction/p3 to avoid confusion with other proteins known as BAFs (Brahma Associated Factors).

We have corrected this oversight in both places.

2. The K6A mutation used to reduce binding to DNA, also reduces/abolishes binding to histones H3 and H1 (Montes de Oca et al., 2005, JBC). This is relevant, and should be considered in the Discussion (e.g., Discussion page 7).

We have addressed this and referenced as suggested, although briefly as discussion is limited.

3. The specific BAF residues affected by the phospho-mimetic MEEEQ construct must be identified on page

5; they are Thr2, Thr3 and Ser4. A nearby phospho-mimetic mutation (K6E) that abolishes binding to DNA, also reduces binding to emerin (Segura-Totten et al., 2002, JCB).

We have added this information about BAF residues where indicated. Reference to K6E was not included sue to space constraints.

4. Briefly discuss (rule out?) whether the BAF mutations used in this study, might also affect binding to lamins A/C (interface includes BAF residues Ala12, Pro14, Phe88, maybe also Asp86; Samson et al., 2018, NAR).

We have added this topic to discussion. We utilized the PDB data from Samson et al., 2018, NAR via PyMol to visualize the locations of the K6A and aa 3-5 (MEEEQ/MAAAQ) mutations and these are not in direct association with the A-type lamin binding surfaces (they project out at an angle from that surface, suggesting the ability of BAF to simultaneously bind DNA, LEM and lamin). Similarly, the L58R mutation is on the opposite side of the protein from the lamin-binding surface where the LEM domain binds.

5. Figure 3B is confusing, unnecessary, and detracts from the manuscript. Overexpressing VRK1 caused an artificial increase in the amount of phosphorylated BAF in the cytoplasm, as detected by a phospho-BAF-specific antibody, but this increased cytoplasmic BAF was not detected by a 'total BAF' antibody. I agree with the authors that native BAF is notoriously difficult to detect via antibodies; this makes Figure 3B inconclusive, so what is the point?

For clarity and rigor and space, we have decided to remove this data

6. Page 6, line 3: clarify which data supports the statement "enrichment [of GFP-tagged LEM domain proteins] was persistent for tens of minutes (Figure 1D)" -- Figure 1D shows the 5 minute timepoint only, for a single LEM protein (LEMD2).

Reference to Fig 1D was a mistake. We have added an image for 20 minutes post-rupture in fig S2A.

7. Page 6, "Similar to GFP-LEM protein recruitment, depletion of BAF... when monitored for up to 15 minutes". Where is 15-minute data shown? (Fig 4 only shows 5-min).

Reference to the time was cut from the text of the results for the sake of space.

8. Page 6, bottom paragraph, a more accurate interpretation of Figure 5 may allow the authors to deduce a timeline for plugging versus repair. E.g.: "but by 5-10 minutes the reporter clearly began to shift back into the nucleus, likely coincident with repair of the rupture (Figure 5A-B, Movie S8)." This interpretation is inaccurate. mCherry-NLS signals in the nucleus look similar at 1-15 min in Fig 5A, suggesting the situation is stable; Fig 5A does not show re-accumulation in the nucleus. Similarly, the 'flatlined' cytoplasmic/nucleoplasmic ratio for the NLS marker with overlapping error bars in Fig 5B suggests that net leakage from the nucleus stops (ie., rupture plugged) within ~6 minutes in wildtype cells. (Fig 5B does not address re-accumulation in the nucleus). When cells lack BAF, leakage appears to continue until ~10-12 minutes.
A longer timeline with constriction-generate ruptures is shown in Fig 5C: control cells appear to rapidly 'plug' the leak, with evidence for partial re-accumulation at the 30 min timepoint (15 min after rupture), and restored nuclear accumulation by ~2 hours. The si-BAF cells are still 'leaking' at 60 minutes (45 min after rupture), and then flatline (achieve 'plug'?) by ~90 minutes, but show no evidence of restored nuclear import.
Revise Discussion accordingly.

The weak signal from the mCherry-NLS reporter may have contributed to our interpretation, therefore we repeated these experiments with the brighter GFP-NLS. In these experiments there is often a leveling off of the cytoplasmic/nuclear ratios, and in other cases there is some evidence of a downward curve suggesting nuclear reuptake. Therefore, to address repair of the ruptures more directly, we photobleached the residual NLG-GFP from the cytoplasm 10 min after rupture and then observed the extent of leakage from the nucleus into the cytoplasm 2 min after photobleaching. In cells without repaired nuclear ruptures (e.g. siBAF) we observed substantive leakage and a decrease in the nuclear GFP-NLS signal. In cells with repaired ruptures (e.g. siControl) we observed no leakage in to the cytoplasm or decrease in nuclear signal. This data is found in Figure S3.

9. Page 6 ("To test how loss of BAF affects nuclear rupture..."): Fig 5C addresses the timecourse of repair, not "rupture". Suggest deleting "Yet unlike with the loss of lamin a/C, the loss of" and changing this final sentence to "We conclude that BAF is uniquely required to repair the NE after nuclear rupture."

This text was removed in the revision to a Report format.

10. Page 7, paragraph 3, "BAF has no substantial intercompartmental mobility" is not true for S-phase: BAF is predominantly nuclear during S-phase, suggesting potent cell cycle regulation of intercompartmental mobility (Haraguchi et al., 2007 JCS).

While we agree that this is relevant information, for the sake of space, we simply stated the following regarding BAF intercompartmental mobility: 'It is known that GFP-BAF, despite its small size, does not passively diffuse through nuclear pores (Shimi et al., 2004)'.

11. Page 7, last paragraph, lines 1-2 do not do justice to your model that BAF binds exposed DNA/chromatin, and then rapidly recruits LEM-domain proteins with their associated membranes. Agree that other proteins may also participate (lamins, and/or other INM proteins). There is direct evidence for substantial 'free' populations of emerin at the NE, based on solubility and non-association with lamins/chromatin (Berk et al, 2013 JBC; Holaska & Wilson, 2007).

Based on our new data on LEM domain recruitment where we clearly are unable to impair the rapid recruitment of LEMD2-GFP by photobleaching of more mobile populations (Fig S2B), it appears that the inherent mobility of these proteins combined with the substantial LEM-domain binding capacity at rupture sites mediated by BAF is sufficient to explain the process. However, for the sake of space there was no discussion on this beyond reporting the finding.

12. Results and Legend for Figure 3C: Which differences were statistically significant?

Statistical differences have been added to the figures and legends.

13. Figure 3C is uninterpretable in black and white. Use larger and distinct shapes (e.g., circles, triangles, squares, open or black) for each construct. Consider moving neighboring error bars slightly to right or left, so they can be distinguished.

This has been corrected for all graphs.

14. Figure 5D (model): Depicting the NE as a single line is unacceptable in the context of this biology. Depict a true NE (inner membrane, outer membrane, thin lumen) in the schematic, and true ER in the bottom panel. The bottom panel should also show that BAF might recruit LEM-proteins in adjacent NE (current panel implies ALL rescuing membrane is from cytoplasm).

We have substantively modified this model, in part to address this critique.

Minor corrections or improvements:

Page 3, paragraph 1: Delete "Within" and in remainder of sentence replace "that is most proximate to the nuclear contents reside a host of transmembrane" with "has many resident transmembrane".

This sentence has been modified as recommended.

Page 3, paragraph 3: "BAF binds to dsDNA as a monomer" is misleading, because BAF forms obligate dimers. A corrected statement, e.g.: "BAF forms obligate dimers, each subunit of which binds dsDNA allowing BAF to 'bridge' two strands of dsDNA".

This sentence has been modified as recommended.

Page 6: Include the measured impact on mechanical force resistance, e.g., "We found that ~20% less force was needed to rupture the nucleus in cells downregulated for either BAF or A-type lamins (Figure S3)." This result is consistent with evidence that BAF strengthens lamin A/C binding to LEM-domain proteins (Samson et al., 2018).

The text has been modified as recommended. Reference to Samson et al., regarding BAF-lamin binding has been included earlier in the manuscript and was not referenced again for space.

Page 7, Discussion paragraph 4, line 3: also cite Samwer et al (2017).

The citation has been added, although its placement in the text has changed due to revisions.

Page 8: Why is cholera toxin added to MCF10A culture medium?

Cholera toxin is a standard component of the culture media for MCF10A cells as indicated by ATCC and others. Apparently, it enhances cAMP and facilitates a more epithelial morphology, at least in some respects.

Legend to Figure 2C: The images are representative of how many total cells examined?

The information has been added to the figure legend.

Legend to Figure 4, line 3: Change "Man1, and Emerin" to "Man1 or Emerin". Line 5: change to "expressing each GFP-tagged... protein"

First point: This has been modified as suggested.
Second point: This sentence was altered in revision.

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

In "Repair of nuclear rupture requires barrier-to-autointegration factor" by Halfmann and colleagues the authors have presented a very novel hypothesis that after nuclear rupture cytoplasmic BAF enters through the hole and binds to exposed chromatin and since BAF can bind to LEM domain nuclear membrane proteins such as emerin it helps to recruit these proteins to the site of rupture to seal the break. This hypothesis is the more attractive since it makes sense with a number of existing data. The microscopy data shows clearly that GFP-BAF targets to the rupture site rapidly and that it can be observed to sequentially

migrate further into the nucleus over time. This latter point in itself would be simple common sense, but the dynamics of the further migration as assessed by the live cell imaging would be further consistent with an initial function at the periphery in rupture sealing. Of note, I was particularly pleased that they used multiple approaches to induce rupture since the first approach with the laser-induced ruptures could have lots of secondary effects on this system and mechanism of repair because of local heating while the pillars bend membranes at non-physiological angles that cause leakage and other though different artefacts so that it is important to show these effects through multiple approaches for inducing rupture. At the same time,

"…their data could also be consistent with a number of other interpretations such as (BAF) initially going against a gradient of other proteins exiting the nucleus that would slow its initial moving further beyond the rupture site and no experiments were done to test this alternate hypothesis such as following proteins at the same time shown in previous studies by other groups studying nuclear rupture and autophagy that show nuclear proteins that appear in the cytoplasm after rupture to determine if their migration out of the cell could be slowing the entry of the BAF up to a certain point.

While we are interested in which proteins move into and out of the nucleus, and which don't, we are focused on BAF for these current studies. The other monitored DNA-binding protein cGAS does enter the nucleus similar to BAF but is restricted to the initial rupture site with no apparent diffusion throughout the nucleus. We attribute this to a differential affinity and off rate of binding for the dsDNA. BAF has nuclear kinases like VRK1 that actively inhibit/reduce the DNA-binding affinity of BAF and would enable an rapid off rate of binding. This also explains why a single amino acid mutation K6A at the DNA (and histone) binding site inhibits this gradual wave of BAF diffusion into the nucleus and instead leads to a rapid diffusion into the nucleoplasm. Similar results are seen for the MEEEQ phosphomimic variant and K6A/L58R mutants. These results would argue against a competing wave of protein leaving the nucleus as these proteins are almost identical to wild type and L58R BAF which do exhibit the wave.

There also were no experiments testing whether BAF has a slightly protracted interaction with these LEM domain proteins or other chromatin proteins in the initial step. FRET could reasonably easily address this issue or FCCS for more depth. While the experiments using BAF mutations is extremely compelling support for the hypothesis, it was not investigated in much detail where doing the FRET or FCCS experiments with the simple microscopy would strengthen this by better clarifying the mechanism.

While these experiments would be interesting, they fall outside the major goals of these studies which are focused on the functional role of BAF at nuclear ruptures. The behavior of BAF at ruptures is certainly of interest to us, although we have not committed the resources to further investigation at this point.

Another mechanistic question that was briefly alluded to by the authors was the potential role of BAF phosphorylation where they tested the effect of VRK1 overexpression, but there were several logical extensions of this experiment missing and outstanding mechanistic questions on this part. For example, what happens when you use a degron to deplete VRK1 after it is no longer required in mitosis? What happens if you block BAF and/or nuclear membrane protein phosphorylation? If a cyto-nuc prep was done to separate cytoplasmic and nuclear BAF, what is the phosphorylation distribution measured in this particular cell line and how effectively does each pool bind to naked DNA, nucleosomes, or nuclear membrane proteins? Is cytoplasmic BAF getting modified once it enters the nucleus and if it is not getting modified then why is it not getting exported once the rupture is sealed? While aspects of BAF nucleocytoplasmic transport remain elusive, the tendency towards further nucleoplasmic migration instead of nuclear export with this pool is interesting and these simple experiments can be done without knowing the details of its import/export pathways.

We have decided to focus our efforts on the functional role of BAF at the nuclear ruptures. Ongoing and future studies are indeed aimed at evaluating the role of various BAF regulators in the behavior of BAF in response to nuclear ruptures, and its functional role in rupture repair.

Probably the most core mechanistic question is does BAF-mediated membrane repair occur through LEM domain membrane proteins that bind BAF as the inner nuclear membrane diffuses to fill the gap or by cytoplasmic vesicles containing newly synthesized nuclear membrane proteins? This could be very simply addressed by separately activating cytoplasmic or nuclear envelope pools of the nuclear membrane protein with photoactivatable GFP shortly before the induced rupture as well as many other approaches including at least for a first approximation +/- cyclohexamide. The methods used to address the timing of membrane recruitment versus LEM domain protein did not yield a clearcut and strong result.

We have tried to address this by evaluating LEMD2-GFP localization in response to rupture under various conditions of photobleaching ER and/or NE populations of LEMD2, including extended photobleaching to remove more mobile population of the protein from being visualized. In all cases, we could not prevent, delay or reduce the relative level of LEMD2 localization to nuclear ruptures suggesting that the inherent mobility of the protein was sufficient to localize it to the massive pool of newly deposited BAF found at a rupture site. (Fig S2B).

The paper itself is clearly written and I was much more pleased than when reviewing most papers with the discussion mostly qualifying the interpretation by limiting it to the results rather than overstating the meaning. In particular it clearly states the need for many of the types of experiments mentioned above before making clear conclusions. This is where my own confusion lies as I would have expected from the results shown for this to be submitted as a Short Report whereas it is listed as an Article. As I think the finding is novel and of import and the data shown are well controlled and ruptures induced by multiple approaches I would support this paper as a Short Report without further experimentation. However, for an Article there is more of an expectation for mechanistic insight and there is very little here and so I would recommend revision with addressing at least one of the two primary questions noted above in detail or both in less depth if it is to be published as an Article.

We do feel that we have provided even more substantive mechanism to these studies in the revision, although we have adjusted the manuscript to a Report format.

Reviewer #3 (Comments to the Authors (Required)):

Review of MS# 201901116, "Repair of nuclear rupture requires barrier-to-autointegration factor" by Halfmann et al.

Summary: Over the past few years there has been rapid progress in defining the molecular details that support repair of nuclear envelope (NE) ruptures in interphase, which appear to be highly related to the closure of the NE at mitotic exit. While previous work has hinted that BAF might be a molecular player in this pathway, this work by Halfmann et al. provides the first solid and thorough investigation of how BAF is functionally important in NE repair. The authors recapitulate previous findings that BAF is recruited to sites of NE rupture, which they introduce through several different mechanisms. They also take on the question of how BAF assembles at sites of NE rupture, providing intriguing evidence for a key role for nuclear compartmentalization of BAF phosphoregulation (in turn linked to DNA binding), with VRK phosphorylating BAF in the nucleus while cytoplasmic BAF is unmodified (and therefore primed to respond by binding to DNA at sites of NE rupture). The authors go on to demonstrate that BAF is essential for the recruitment of several LEM domain proteins to sites of nuclear rupture (namely emerin, LEMD2 (LEM2), and MAN1). Consistent with previous work suggesting that LEMD2/LEM2 is particularly critical for NE repair and/or closure of the NE at mitotic exit, the authors demonstrate that BAF depletion strongly inhibits

reestablishment of the nuclear envelope barrier after rupture.

Assessment: Overall this is an interesting, timely, well-conceived and convincing study that provides new insights into the mechanisms governing NE repair. The data are of high quality and the paper is well-written and accessible. The authors are generally careful in their interpretations and, taken together, the observations make a nice story. As outlined below, my only criticisms focus on 1) the need to provide more quantitative analysis of some phenotypes, particularly the dynamics of BAF mutants; and 2) the somewhat glaring lack of discussion of the ESCRT machinery and other insights that have come from the study of NE closure at mitotic exit that are really critical to put this work into context. In addition, comparing the effects of BAF depletion with that of the established NE closure machinery (LEM2/CHMP7) would also provide deeper insight. While these are relatively minor issues, the effort to address them would improve the manuscript and its impact.

Specific Points:

1. The authors describe a clear difference in the recruitment of the DNA binding, LEM domain protein binding and phosphomimetic mutants of BAF compared to the WT. From the stills (Fig. 3A) and movies the described differences were not qualitatively obvious. The data may well support the authors' interpretation, but it would be very helpful to have some quantitative analysis to back up their statements (e.g. fluorescence intensity of BAF at the rupture, kinetics of accumulation and dissolution?). There is quantification of the nuclear exchange (Fig. 3C), but this comes later and it's not clear whether this reflects the affinity of the non-specific DNA binding and/or the off-rate from the accumulation at the rupture.

We have adjusted our analysis of the BAF entry into the nucleus to reflect a ratio of its localization at a site proximate to the rupture compared distal to the rupture. Hopefully this provides some more clarity to the point that the WT BAF behavior during entry is largely being dictated by its DNA/histone binding properties.

2. Given that the authors place BAF as critical to the recruitment of LEM domain proteins including LEM2/LEMD2, it seems very strange that they do not discuss the model built up over the past few years that LEM2 and its orthologues (from budding and fission yeasts) promote NE repair by recruiting the ESCRT-III machinery (Gu et al., PNAS, 2017; Webster et al., EMBO J, 2016). The role for ESCRTs also plays a prominent role in the Denais and Raab work. Without this piece the model seems incomplete, as one would expect that BAF is the far up-stream factor that ultimately engages the ESCRT machinery. Moreover, this work raises issues that really do warrant discussion in this manuscript (next point).

3. LEM2 was found to be critical for recruitment of the ESCRT machinery at mitotic exit (Gu et al). The authors' findings that BAF promotes recruitment of all tested LEM domain proteins raises the question of whether there is specificity or redundancy. Given the very strong defect in reestablishing the NE barrier in BAF depleted cells (Fig. 5), it would be very helpful to know how depletion of only LEM2 or CHMP7 would quantitatively compare. Although this is a relatively small experiment, it would go a long way to shed light on whether all the LEM proteins are functionally relevant in the NE repair pathway, which remains an open and important question.

Response to points 2 and 3 combined. We agree that these experiments are worth doing and thus have made efforts to show that Chmp7 recruitment to nuclear ruptures is LEMD2, and thus BAF dependent. We have also shown that loss of Chmp7 or LEMD2 similarly do not substantively alter the eventual repair of a ruptured nucleus. However, the simultaneous depletion of LEMD2, Emerin and Ankle2 does prevent the repair of nuclear ruptures similar to the loss of BAF. This suggests that the primary mechanism of BAF-mediated repair of nuclear ruptures is to recruit transmembrane LEM-domain proteins and thus membranes to the rupture site. And that there must be ESCRT-III independent mechanism to reseal those membranes.

We have ongoing studies to explore those other mechanisms and to ascertain if there is functional redundancy or primacy in their involvement in the repair process.

May 8, 2019

RE: JCB Manuscript #201901116R

Dr. Kyle J Roux
Sanford Research/USD
2301 N 60th St. East
Sioux Falls, SD 57104

Dear Dr. Roux,

Thank you for submitting your revised manuscript entitled "Repair of nuclear ruptures requires barrier-to-autointegration factor". Thank you for your patience as we editorially assessed the revision and thank you for your efforts to address the issues shared in review. Based on our evaluation, we find that you have thoroughly addressed all the pertinent points of the reviewers and we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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Suggested eTOC: (edited to fit our style guide)
Halfmann et al. describe a role for the protein BAF in the repair of nuclear envelope ruptures. BAF recruits some LEM-domain proteins and membranes to the sites of nuclear rupture, yet surprisingly, ESCRT-III is not required to repair ruptures. These results provide new insight into the mechanisms of nuclear envelope repair.

2) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to 5A.
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis. Please add molecular weight with unit labels on the following panels: S1BCD

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