A septin-Hof1 scaffold at the yeast bud neck binds and organizes actin cables

Mikael Garabedian, Alison Wirshing, Anna Vakhrusheva, Bengi Turegun, Olga Sokolova, and Bruce Goode

Corresponding author(s): Bruce Goode, Brandeis University

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Transaction Report:

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RE: Manuscript #E19-12-0693
TITLE: A septin-Hof1 scaffold at the yeast bud neck spatially organizes actin cables

Dear Prof. Goode:

Thank you for submitting your work "A septin-Hof1 scaffold at the yeast bud neck spatially organizes actin cables" to MBoC.

Your paper has been reviewed by two experts in the field as well as by myself. As you will see from their comments appended below, they diverge somewhat in their opinion of the work. The first reviewer is overall positive with three major technical points to be addressed experimentally and a few minor points that can all be addressed by text changes and/or quantification of existing data (I don't feel minor point 5 needs to be addressed experimentally). The second reviewer is more negative, remarking on the overlap between the first part of your current manuscript and published data from your lab, but is more enthusiastic about the super-resolution imaging in the second part, which they however finds too preliminary. The second reviewer raises the important point that your genetic analysis of cable organization in hof1Δ bnr1Δ single and double mutants is contradictory with your previous study (Graziano et al, MBoC 2014 - Figure 2A-B showed that "Deletion of BNR1 sup¬pressed the misoriented cable phenotype caused by hof1Δ"). This controversial dependence on bnr1Δ should be explained and openly discussed in the text. S/he also makes two additional valid points of control.

My reading of your manuscript is that the principal novel element comes from the identification of direct actin binding and bundling of the Hof1 linker region, which, in contrast to the Hof1 functions you previously described, underlies a Bnr1-independent actin cable organization function. I feel that this finding is important and should be published, but I also agree with reviewer 2 that this main conclusion is at present not sufficiently supported by in vivo experiments. The entangled cable phenotype observed in hof1Δlinker is similar to that observed in hof1ΔN or hof1ΔC alleles, and so is not sufficient as evidence for the role of Hof1 actin binding in vivo. To support the model proposed in Figure 7, it would be necessary to extend the super-resolution imaging to test the organization of actin cables relative to septins in the hof1Δlinker allele, as proposed by reviewer 2, and to bnr1Δ cells, to support the Bnr1-independence of this Hof1 function. Comparison of these mutants with wildtype may also require some quantitative comparison of actin cable-septin alignment.

Finally, it came to my attention that your Figure 4A has two identical images (those placed under hof1Δ bnr1Δ and under hof1Δlinker bnr1Δ labels). I trust this is a mistake during figure construction, which will have to be corrected.

I look forward to seeing a revised version of your manuscript.

Sincerely,

Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Goode,

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.

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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):

In their manuscript, Garabedian et al. impressively combine genetics, SIM, live cell confocal imaging, TIRF imaging of purified proteins, and electron microscopy to show how the FBAR protein Hof1 participates in actin cable organization in cooperation with formins and septins in budding yeast. This work builds on the results shown in their 2015 J Cell Biol paper. We appreciate the efforts that have been made to quantify the phenomena they observe. Overall the work is comprehensive and we feel that, provided they address the comments below, it merits publication in Molecular Biology of the Cell.

Major points:

1. The direct binding between the Hof1 linker domain and actin is a critical result in this paper. However, the TIRF assay used to demonstrate actin binding was unconvincing. Measuring CV of the entire TIRF field is a rather indirect measure of actin binding and did not distinguish binding from bundling. As a result, the actin-binding capability of the N-terminal domain remains ambiguous. Furthermore, does the monomeric linker domain bind actin? A more direct binding assay, such as actin pelleting, would be much more convincing and allow an estimate the affinity between the monomeric Hof1 linker domain and actin, and between the Hof1 N-terminal domain and actin.

2. The Hof1Δlinker cells show a loss of function. However, the authors have not reported whether the expression of hof1Δlinker is similar to wild type. The authors should compare the total expression level of this construct compared to wild type Hof1-GFP, either with a Western blot or measuring total GFP intensity per cell.

3. Figure 5A shows that Hof1 links septins to actin. However, in the image shown, the actin filaments are not bundled and the septins appear as aggregates rather than filaments. Is this due to the concentration of actin used or the order in which the proteins were mixed? The authors should justify the appearance of septins and actin in this micrograph, or otherwise change the order of addition of protein (e.g. actin + Hof1, followed by septin) to more convincingly show the mutual association between actin filaments, Hof1, and septins.

Minor points:

In the bar graphs, for example in Figure 1, changes in variability are equally interesting to changes in the mean value. However this information is hidden the bar graphs with SEM error bars. Can the authors show all the data and represent error bars as standard deviations?

In order for us to evaluate the efficacy of the "matlab software" used to measure cable extension rate and angle, The authors should include supplemental movies of cells used for the tracking results in Figure 2 (cable growth and vesicle trajectories). What does the algorithm do? Does it track and report positions? It is not sufficient to say that software was simply used.

The coefficient of variation measurements are not well-described. Are the measurements always of segmented cable intensity? Or of entire cells?

Describing the septin complexes simply as "septins" was oversimplifying for a specialist journal like MBoC. In the legend and or results section, when introducing the septin construct, please list the proteins in the "septins" complex.

The electron micrographs in Figure 3D are intriguing. We would appreciate it if the authors showed

larger fields of these EM results of Hof1+actin in the supplement. The filaments may bundle somewhat in the control situation, because of the use of acidic uranyl acetate for contrasting. Negative staining with neutral solutions of phosphotungstic acid or sodium silicotungstate might have better separated the filaments in the control (like in Resch et al., Journal of Structural Biology 137 (2002) 305-312), to show a larger bundling effect when Hof1 is added, as is shown in their TIRF assays.

The authors state based on their EM data that "Hof1 particles bound to actin in a regular pattern." What does "regular" mean here? They should clarify that and maybe add some quantification for the regularity (e.g. distribution of the distance between individual Hof1s along the filaments and on parallel filaments.

Authors should check the scale bar of the average (fig 3E). In the text they say the structure is about 20nm in length, but the scale bar suggests it is about 10 or 12 nm.

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This manuscript follows two previous research articles from the same lab on Hof1. The first manuscript (Graziano et al., Mol Biol Cell, 2014) reported a physical interaction between Hof1 and the formin Bnr1, in which the SH3 domain of Hof1 tunes Bnr1's actin nucleation activity and actin cable formation in cells. The second manuscript (Garabedian et al., Nat Cell Biol, 2018) described the function of Hof1's F-BAR domain in actin cable organization.

I have mixed feelings about the current manuscript because the first half of it is similar to previously published results (see point 1/). The second half provides some original and interesting results but which are unfortunately too incomplete to propose a convincing model for Hof1-Septin's function in actin cable organization (see point 2/). For these reasons, I am unfortunately not supportive of a publication of this manuscript.

Main comments:
1/ The first part of the manuscript on Hof1 requirement for proper spatial organization of actin cables has pretty much been published in the 2014 Graziano paper (see for example Fig.1H or Fig.2A of that paper), and I do not see why it requires again such a long description and two new Figures in this manuscript.

Moreover, I am quite surprised that the analysis of actin cables in wild-type, hof1n, bnr1n and hof1n bnr1n cells in the Graziano paper (Fig.2B) is reaching an opposite conclusion to the current manuscript. In this previous paper, the conclusion was that deletion of Bnr1 suppressed the misoriented cable phenotype caused by hof1n, which the authors acknowledged page 1733. In this new manuscript, the conclusion is opposite but the authors do not explain these discrepancies.

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2/ I am more enthusiastic about the super-resolution images showing actin cables apparently

emerging from a regular scaffold of septins and Hof1. This is a beautiful result, but which does not demonstrate on its own that such scaffold is required to organize spatially actin cables. It would be for example more convincing to use this technique to show whether hof1nlinker cells have a proper localization of Hof1 and Cdc3 at the bud neck but an abnormal co-alignment of actin cables at the bud neck.

Other comments:
1/ There is at least one paper from the Lecuit lab showing that actin and septin filaments can interact and co-align in the absence of additional proteins. The authors should discuss whether the absence of interaction in this paper comes from the use of a different protocol or from an absence of interaction between the two yeast proteins.

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We thank the reviewer for these suggestions. We provided one demonstration of F-actin binding through direct visualization, by showing that labeled full-length Hof1 associates with actin filaments in TIRF assays (Figure 3A). We also attempted high speed co-pelleting assays, but found that Hof1 is prone to pelleting on its own at high *g* forces. This is common for large multimeric proteins with unstructured domains, including many BAR family proteins that we have worked on previously (e.g., Syp1), which have a tendency to self-associate with time and sediment on their own in ultracentrifugation assays. This can sometimes be troubleshot, usually after much effort, by identifying specific biochemical conditions (e.g., including specific salt concentrations and detergents) that prevent self-association without compromising F-actin binding. However, this would be quite challenging for us to carry out under the circumstances of the COVID outbreak. Finally, we note that full-length Hof1 and several of its subfragments were able to bundle F-actin, as seen clearly in the TIRF images, and confirmed by EM and CoV analysis (Figure 3B-E). Bundling requires binding to F-actin, and our direct visualization of labeled Hof1 on F-actin (Figure 3A) confirms the association. Based on these arguments, we stand by our conclusion that Hof1 binds F-actin.

2. The Hof1Δlinker cells show a loss of function. However, the authors have not reported whether the expression of hof1Δlinker is similar to wild type. The authors should compare the total expression level of this construct compared to wild type Hof1-GFP, either with a Western blot or measuring total GFP intensity per cell.
We managed to complete this experiment just before the lab shut down. The data appear in a new Supplemental Figure, and show that the total cellular levels of the two integrated constructs (Hof1-GFP and Hof1Δlinker-GFP) are similar (Figure S3B). Thus, the actin cable phenotypes in *hof1Δlinker* cells (which partially mimic *hof1Δ*) do not arise from lower expression levels of Hof1Δlinker, or an inability of this construct to localize to the neck. We also note that Hof1Δlinker-GFP signal at the neck was on average about two-fold higher than Hof1-GFP at all stages of bud growth (Figure S3C). The reason for this is not known, but may stem from the linker region being a target of signaling pathways that help regulate Hof1 localization. In addition, in some small-budded cells, we observe Hof1Δlinker-GFP signal at the bud tip in addition to the neck, again pointing to the linker domain having some influence on Hof1 localization. Importantly, this partial mislocalization was not observed in medium- and large-

budded cells, where we continued to observe cable organization defects caused by *hof1Δlinker*. Finally, we have added a statement in the paper to more cautiously interpret our *hof1Δlinker* results (page 7): "Importantly, our data do not rule out the possibility that additional molecular interactions of the linker region, beyond F-actin binding, could contribute to actin cable organization."

3. Figure 5A shows that Hof1 links septins to actin. However, in the image shown, the actin filaments are not bundled and the septins appear as aggregates rather than filaments. Is this due to the concentration of actin used or the order in which the proteins were mixed? The authors should justify the appearance of septins and actin in this micrograph, or otherwise change the order of addition of protein (e.g. actin + Hof1, followed by septin) to more convincingly show the mutual association between actin filaments, Hof1, and septins.
It is more evident in the movies from which the still images in Figure 5A are taken, that the actin filaments become bundled by Hof1 over time. Therefore, we have added a movie to help clarify this point (see Video S1). In our TIRF experiments, the control septin filaments (formed by Cdc3, Cdc10-SNAP, Cdc11, Cdc12) in the absence of Hof1 have a similar appearance, and lengths, to those described in the original paper (Renz et al, 2013, *BMC Biotechnology*; for example see Fig 2 in this paper). To make all of this more clear, we have changed Figure 5A to show a closer-up view of the actin and septin filaments. The addition of Hof1 thickens the septin filaments (which the reviewer thought might be aggregates). As suggested by our EM data (Figure S4), this is likely due to Hof1 dimers crossbridging (bundling) septin filaments. This is also consistent with earlier in vivo studies reporting that Hof1 overexpression thickens septin structures (Oh et al., 2013; *Mol. Biol. Cell*; Lippincott and Li, 1998; *J. Cell Biol.*).

Minor points:

In the bar graphs, for example in Figure 1, changes in variability are equally interesting to changes in the mean value. However this information is hidden the bar graphs with SEM error bars. Can the authors show all the data and represent error bars as standard deviations?
This was a great suggestion. We now have changed Figure 1, and most of the other graphs in the paper, to show all of the data points and SD (Figures 1E-H, Figure 2B,C, Figure 4B-D, Figure 5D-F, and Supplemental Figure S1B).

In order for us to evaluate the efficacy of the "matlab software" used to measure cable extension rate and angle, The authors should include supplemental movies of cells used for the tracking results in Figure 2 (cable growth and vesicle trajectories). What does the algorithm do? Does it track and report positions? It is not sufficient to say that software was simply used.
In the Methods section, we have added clarification of what the Matlab program does and what the user does in this analysis: (1) user designates the boundaries of the cell and the mother-bud axis, (2) user then designates starting and end points for each cable (from the movie), and (3) program calculates cable velocity and extension angle (relative to the mother-bud axis). Further, we reference example movies of the cable analysis in our previous paper (Eskin et al., 2016; *Mol. Biol. Cell*).

The coefficient of variation measurements are not well-described. Are the measurements always of segmented cable intensity? Or of entire cells?
It is always a measure of the entire cable network in the mother compartment, and never segmented cable intensity. We clarified this in the Methods by saying, "*The CoV measurements are made by first tracing the outline of the mother cell compartment in ImageJ, and then measuring the mean fluorescence of actin cable staining and the standard deviation. The CoV is*

a ratio of the standard deviation over the mean. Measurements of wild-type cells with well defined, brightly stained cables against a dark cellular background produces a high standard deviation and thus a higher CoV. Measurements of cells with more disorganized and dispersed cable networks result in lower stand deviation values and, consequently, a lower CoV."

Describing the septin complexes simply as "septins" was oversimplifying for a specialist journal like MBoC. In the legend and or results section, when introducing the septin construct, please list the proteins in the "septins" complex.
We fixed this in the Results, clarifying that the septin filaments we are working with here are formed from: Cdc3, Cdc10-SNAP, Cdc11, and Cdc12.

The electron micrographs in Figure 3D are intriguing. We would appreciate it if the authors showed larger fields of these EM results of Hof1+actin in the supplement. The filaments may bundle somewhat in the control situation, because of the use of acidic uranyl acetate for contrasting. Negative staining with neutral solutions of phosphotungstic acid or sodium silicotungstate might have better separated the filaments in the control (like in Resch et al., Journal of Structural Biology 137 (2002) 305-312), to show a larger bundling effect when Hof1 is added, as is shown in their TIRF assays.
As suggested, we now show wider fields of view EM images for control (F-actin alone) and F-actin + Hof1 (Figure 3D). These images are representative of what we see all over the EM grids, and are consistent with our TIRF analysis of Hof1 bundling effects (Figure 3A and 3B).

The EM images reveal that Hof1 induces organized bundles of actin filaments with crossbridges (which appear like railroad ties) connecting adjacent filaments (Figure 3E-G). These crossbridges even have the same shape of elongated Hof1 dimers that we defined previously using single particle EM (Garabedian et al., *2018 J. Cell Biol*). The confusion in this figure may have come from our attempt to compare the organized Hof1-induced actin bundles to the less organized, non-specific actin bundles occasionally found on the control grids (these are more rare, but they can be found, likely due to the negative stain effects the reviewer mentions). The 'control bundles' have a very different appearance from Hof1-induced bundles; they are not nicely aligned, and they lack visible crossbridges. They appear to be non-specifically entangled actin filaments. In light of the confusion it caused, we removed this comparison from the paper, and replaced it with the images now shown in Figure 3D-E.

The authors state based on their EM data that "Hof1 particles bound to actin in a regular pattern." What does "regular" mean here? They should clarify that and maybe add some quantification for the regularity (e.g. distribution of the distance between individual Hof1s along the filaments and on parallel filaments.
We have clarified this in the Results, and added quantification of the distances separating crossbridges (Figure S2B). We meant that we see regularly-spaced crossbridges, which have a similar elongated rod-like shape and appearance, linking adjacent filaments in Hof1-induced actin bundles (Figure 3E). These crossbridges run perpendicular to the actin filaments.

Authors should check the scale bar of the average (fig 3E). In the text they say the structure is about 20nm in length, but the scale bar suggests it is about 10 or 12 nm.
We thank the reviewer for catching this mistake. The scale bar is indeed 20 nm, now corrected.

Reviewer #2 (Remarks to the Author):

This manuscript follows two previous research articles from the same lab on Hof1. The first manuscript (Graziano et al., Mol Biol Cell, 2014) reported a physical interaction between Hof1 and the formin Bnr1, in which the SH3 domain of Hof1 tunes Bnr1's actin nucleation activity and actin cable formation in cells. The second manuscript (Garabedian et al., Nat Cell Biol, 2018) described the function of Hof1's F-BAR domain in actin cable organization.

I have mixed feelings about the current manuscript because the first half of it is similar to previously published results (see point 1/). The second half provides some original and interesting results but which are unfortunately too incomplete to propose a convincing model for Hof1-Septin's function in actin cable organization (see point 2/). For these reasons, I am unfortunately not supportive of a publication of this manuscript.

Main comments:
1/ The first part of the manuscript on Hof1 requirement for proper spatial organization of actin cables has pretty much been published in the 2014 Graziano paper (see for example Fig.1H or Fig.2A of that paper), and I do not see why it requires again such a long description and two new Figures in this manuscript.
We believe that this new body of work includes many novel results, and has minimal overlap with our previous work on Hof1. We appreciate that at first glance this paper may appear to revisit old territory; this may be our fault for not making it more clear in the writing. In the revised text, we have clarified this point (Abstract, Introduction, and beginning of Results).

Our previous work on Hof1 focused exclusively on its genetic and biochemical roles in regulating the formin **Bnr1** to influence actin cable architecture and function. In the present manuscript, we instead discover and characterize Bnr1-independent roles of Hof1 in actin cable organization. These effects of Hof1 on cable organization (in the absence of *BNR1*) were not noticed in our earlier work (Graziano et al., 2014) likely because of the poorer quality of imaging analysis at the time. In the new work, we have used superresolution (SIM) imaging and quantitative cable analysis (SOAX) techniques, both of which were not available to us before the 2014 study. Our data using these improved methods clearly show that loss of *HOF1* alters actin cable organization even in a *bnr1*Δ background. This novel observation is established by the data presented in Figures 1 and 2. After that, we delve deeper into the mechanistic basis for these Bnr1-independent functions of Hof1, and discover that Hof1 directly binds and bundles F-actin in vitro (mediated in large part by its linker domain), and can link septin and actin filaments in vitro (Figures 3-5). Finally, we use superresolution (SIM and Airyscan) microscopy to show that Hof1 and septins form novel striations at the bud neck - running parallel to the mother-bud axis - and that actin cables align with these septin-Hof1 striations. All of these findings are novel, and have not been reported before, by our lab or others.

Moreover, I am quite surprised that the analysis of actin cables in wild-type, hof1n, bnr1n and hof1n bnr1n cells in the Graziano paper (Fig.2B) is reaching an opposite conclusion to the current manuscript. In this previous paper, the conclusion was that deletion of Bnr1 suppressed the misoriented cable phenotype caused by hof1n, which the authors acknowledged page 1733. In this new manuscript, the conclusion is opposite but the authors do not explain these discrepancies.
We clarify this apparent discrepancy in the revised manuscript. The key points are as follows: (1) In our previous study, we showed that *bnr1*Δ partially (not fully) suppresses *hof1*Δ defects in cable organization. Thus, we have always known that there may be Bnr1-independent functions of Hof1 in actin cable organization. (2) As mentioned above, our current study uses improved

super-resolution structured illumination microscopy (SIM), which reveals finer details of cable staining. This approach was more conclusive in revealing actin cable defects in *hof1Δbnr1Δ* cells, which were only hinted at previously. Third, in our current study we have performed a more quantitative, unbiased analysis of cable defects (using SOAX); these methods were not available to us before our 2014 study.

Overall, these data bring confusion to the readers because it seems that different metrics chosen by different authors (even from the same lab) to analyze actin cables leads them to reach opposite conclusions. I am wondering in the end whether any strong conclusion can be made from an analysis of actin cable orientation at the whole cell level for proteins like Hof1 and septins which are themselves localizing at the bud neck.
Above we explain that the methods have improved, allowing new observations and discoveries. As expected, techniques improve over time, which has allowed us to make new discoveries about Hof1's functional roles at the bud neck in actin cable assembly and organization. Our study shows that Hof1 (which is anchored to septins at the neck) is required to align cables with septin striations. Actin cables emerge from the striations and grow into the mother compartment. One of the most novel aspects of our findings is that they offer a new paradigm, in which actin regulatory proteins can be 'patterned' at the cell cortex to control spatial organization of cellular actin networks. Consistent with this view, in *hof1Δ* cells, we see defective cable extension angles (through the neck into the mother) and cable entanglement in mother cells. Further, Hof1 links septin and actin filaments in vitro.

2/ I am more enthusiastic about the super-resolution images showing actin cables apparently emerging from a regular scaffold of septins and Hof1. This is a beautiful result, but which does not demonstrate on its own that such scaffold is required to organize spatially actin cables. It would be for example more convincing to use this technique to show whether hof1nlinker cells have a proper localization of Hof1 and Cdc3 at the bud neck but an abnormal co-alignment of actin cables at the bud neck.
We now show that Hof1Δlinker-GFP localizes to the neck (Figure S3), but we would need to build a new strain (Cdc3-Apple, hof1Δlinker-GFP) and image it by SIM to confirm that it is patterned in striations like Hof1-GFP. Unfortunately, we did not finish making the new strain before the lab was shut down due to the COVID-19 outbreak. However, we strongly predict that the Hof1Δlinker-GFP construct would localize to the septin striations given that it has its CC2 domain, which binds septins and is the chief determinant of Hof1 localization to the neck (Oh et al., 2013; *Mol. Biol. Cell*).

Other comments:
1/ There is at least one paper from the Lecuit lab showing that actin and septin filaments can interact and co-align in the absence of additional proteins. The authors should discuss whether the absence of interaction in this paper comes from the use of a different protocol or from an absence of interaction between the two yeast proteins.
We now highlight these differences in our Discussion. The Lecuit study showed that *Drosophila* septins directly bind and align with F-actin. We do not necessarily expect *S. cerevisiae* septins to behave the same way. Different septins (even from the same species) can have highly distinct binding partners, and can differ tremendously in their interactions with microtubules and actin. For example, some mammalian septins (such as SEPT9) bind to microtubules and others do not, because they contain specific sequences that mediate microtubule binding. Our analysis on *S. cerevisiae* septins shows that they do not directly bind and align with actin filaments, but that Hof1 (which binds to F-actin and septins) can mediate their interaction.

2/ I think that it would be important to control with a pyrene assay whether Hof1nlinker protein inhibits Bnr1 with the same efficiency than wild-type Hof1.

We do not make any claim that the linker domain functions exclusively in F-actin binding. In fact, in our Graziano et al., 2014 (*Mol. Biol. Cell*) there was some evidence that the linker region may contribute to Bnr1 inhibition. Given the length of the linker region, it would not be surprising if it had additional molecular interactions besides F-actin. We now mention this in the Results where we discuss Figure 4. Importantly however, our comparison of actin cable defects in *hof1Δlinker bnr1Δ* versus *bnr1Δ* cells demonstrates that the cable defects caused by *hof1Δlinker* are not suppressed by *bnr1Δ* (Figure 4), and therefore cannot be due to Hof1 inhibitory effects on Bnr1.

RE: Manuscript #E19-12-0693R
TITLE: "A septin-Hof1 scaffold at the yeast bud neck binds and organizes actin cables"

Dear Bruce,

Your additions and re-write have made the story much clearer. It will be a nice addition to the field. Please note that I noted some typos in figure calling in the text (for instance Fig S3 mislabeled as S2).

Best wishes,
Sophie

Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Goode:

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