EMBO reports

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Bin1 directly remodels actin dynamics through its BAR domain

Nina M Dräger, Eliana Nachman, Moritz Winterhoff, Stefan Brühmann, Pranav Shah, Taxiarchis Katsinelos, Steeve Boulant, Aurelio A Teleman, Jan Faix, Thomas R Jahn

Corresponding author: Thomas Jahn, German Cancer Research Center

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 29 March 2017

Thank you for the submission of your research manuscript to our journal. We have now received a complete set of reviews form all referees, which I include below.

As you will see, two referees support publication of your manuscript in EMBO reports. Referee 3 however, who is an expert in actin cytoskeleton organization, considers the manuscript unsuited to publication and raises a number of important concerns regarding the conclusiveness of the data.

Thank you also for the submission of a point-by-point response to the reviewers comments. I think that the localization of Bin1 to actin filaments/bundles should be at least evaluated with fluorescent antibodies. To complete the description of the actin binding of Bin1 it should be tested if Bin1 also binds to monomeric actin, as you outlined (quantify Bin1 in SN fraction or perform pull-down assays). The control experiment for Fig. 2E without actin should be supplied to rule out any doubt. I understand the difference to the observed binding of the SH3 domain to actin can originate from different conditions or different Bin1 isoforms. Nevertheless, I think that it will be important to address this point and to test binding between actin and the SH3 domain using your experimental conditions. Please also address point 3 and 4 of referee 3. Regarding the in vivo data, I agree that these are limited since you knocked down full length Bin, but, as I already indicated, it will not be essential to expand this part.

In summary, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point

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response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Regarding data quantification, please reassure that the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is specified in all figure legends.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
- a separate PDF file of any Supplementary information (in its final format)

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

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REFEREE REPORTS

Referee #1:

Drager et al., demonstrated a novel function of BAR domain protein Bin1 in bundling actin and preventing them from depolymerisation using a combination of in vitro imaging and biochemical assays. They further demonstrated that this novel function of Bin1 could be applicable to tau-stablized actin bundles with both in vitro and in vivo loss-of-function evidences. Overall, I think that the findings are interesting, the study is well executed and the figures are clearly organized with proper statistical analysis. The manuscript could thus be accepted with minor revisions.

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Major comment:

While I find the experiments to be largely convincing, one key experiment missing from the manuscript is the localization of Bin1 in either the in vitro assays (either fluorescent assays like Fig 4A or EM such as Fig 1c), or the in vivo studies (Fig 6A). The closest might be in Fig 1C where some eletron-dense material was pointed out. Does the author imply that Bin1 decorate the filament at sparse loci along the filament? Is it possible to use fluorescently labelled Bin1 in the in vitro system or GFP-fusion protein in the in vivo system? Localization of the Bin proteins, ideally also the dose-experiment, could be one-step closer to demonstrate whether Bin1 proteins are polymerizing along the filament, and how they may promote actin bundling.

Minor comments:
1. I think the evidence supporting the interaction between Bin1 and actin is compelling. But strictly speaking, I am not sure whether the author could conclude that Bin1 preferentially binds to F-actin. In Fig 1B, it looks like the relative ratio of Bin1 in solution vs. pellet is similar to the ratio of actin in solution vs. pellet. It will be great if the band intensity could be quantified. Similarly, Fig 1D could also be explained if Bin1 bind to actin in the monomeric form. The distinction may not be critical for the main conclusions of the paper but it will have important implications with regard to Bin1's binding site on the actin.
2. BAR proteins tend to aggregate at low salt conditions, i.e. the conditions where the authors perform the actin polymerization assays. I am wondering whether this could affect Fig 2E/F.
3. The fittings in Fig 3C do not visibly match with the original data.
4. In light of findings in Fig 4A, are the EM image in Fig 1C in fact an actin bundle? If not, did the authors take a look at the ultrastructure of the EM bundles formed using the conditions of Fig 4A?
5. Page 10, line 10, should be "to some extent".

Referee #2:

Here the authors demonstrate that BIN1 can bind actin and alter actin dynamics in vitro and probably in vivo. While it is not terribly surprising that BIN1 binds actin through its BAR domain since BAR domains in other proteins are known to bind actin, demonstration of actin binding and in vitro investigation of the effects on actin polymerization and bundling are important results. Since BIN1 has been linked to Alzheimer's disease and previous efforts to investigate the potential disease role of BIN1 have mostly been to link BIN1 with Abeta through APP processing, a mechanistic focus on BIN1 and tau is welcome and likely to be of interest to a broad audience. The manuscript does not go very far in probing the in vivo interactions of BIN1, actin and tau; however, the focused nature of the manuscript fits with the scope of EMBO Reports.

A recent study in Developmental Cell (2015;35:186) suggests that the SH3 domain, not the BAR domain interacts with actin. The authors should comment.

Referee #3:

Summary:
This manuscript investigates the F-actin bundling properties of Bin1 (amphiphysin2). Bin1 has a N-terminal N-BAR domain and a C-terminal SH3 domain. The N-terminal domain binds to curved lipid membranes, while the SH3 domain has multiple interactions with other proteins, including some actin regulators.
The authors report that Bin1 binds weakly to F-actin, inducing the bundling of actin filaments. The authors provide results suggesting that such interaction occurs through the BAR domain. F-actin bundling by Bin1 provides a weak resistance to depolymerization in the presence of ADF/cofilin. This effect is conserved when actin bundles are simultaneously decorated by tau. As a consequence, the authors suggest that Bin1 could be an interesting target in taupathies, where an accumulation of actin rods in brain cells could be limited by a downregulation of Bin1.

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This manuscript suffers from a number of problems listed below.

1/ D'Alessendro et al. (Dev Cell, 2015) already report the binding of Bin1 from C. elegans to F-actin. Therefore, the results from Figure 1 are not as novel as it is suggested.

2/ The same study (D'Alessendro et al.) demonstrates clearly that the interaction between Bin1 and F-actin depends on its SH3 domain. In this current manuscript, using protein crosslinking and mass spectrometry analysis, the authors reach the opposite conclusion that the interaction is mediated through the BAR domain.
The authors need to explain why their result is different, and to provide data indicating the binding affinity of various Bin1 constructs to F-actin.

3/ Binding of BAR domain proteins to F-actin is often attributed to non-specific interactions between highly-charged membrane binding surfaces and a charged polymer. In the case of Bin1, a comparison of Figure 1D and Figure 2D indicates that the affinity of the BAR domain for F-actin is much weaker that the FL construct. Overall, the biological relevance of this interaction should be carefully questioned in vivo with specific point mutants to rule out any misinterpretation.

4/ Pyrene Assays are not convincing and misinterpreted. Why do actin assembly curves always drop for 20 min before showing a sudden increase?
I also disagree that the apparent increase in the rate of actin assembly in the presence of cofilin and Bin1 could be due to a stabilization of actin filaments against depolymerization. In a pyrene actin assembly assay, depolymerization is always negligible. An enhanced severing of actin filaments is more likely to explain this result.

5/ Figure 6. The authors are lowering the expression of a full-length Bin1 construct. Given the fact that the SH3 domain of Bin1 interacts also with various actin regulators, it is difficult to conclude from this experiment that Bin1's bundling activity is what stabilizes tau bundles. The authors need to provide additional experiments confirming that BAR domain bundling activity is responsible of this observation.

1st Revision - authors' response 03 July 2017

Point-by-point response to the referees' comments

Referee #1

Drager et al., demonstrated a novel function of BAR domain protein Bin1 in bundling actin and preventing them from depolymerisation using a combination of in vitro imaging and biochemical assays. They further demonstrated that this novel function of Bin1 could be applicable to tau-stablized actin bundles with both in vitro and in vivo loss-of-function evidences. Overall, I think that the findings are interesting, the study is well executed and the figures are clearly organized with proper statistical analysis. The manuscript could thus be accepted with minor revisions.

Major comment:

While I find the experiments to be largely convincing, one key experiment missing from the manuscript is the localization of Bin1 in either the in vitro assays (either fluorescent assays like Fig 4A or EM such as Fig 1c), or the in vivo studies (Fig 6A). The closest might be in Fig 1C where some eletron-dense material was pointed out. Does the author imply that Bin1 decorate the filament at sparse loci along the filament? Is it possible to use fluorescently labelled Bin1 in the in vitro system or GFP-fusion protein in the in vivo system? Localization of the Bin proteins, ideally also the dose-experiment, could be one-step closer to demonstrate whether Bin1 proteins are polymerizing along the filament, and how they may promote actin bundling.

We thank the reviewer for supporting our main conclusion and raising this additional interesting point. We addressed the localization of Bin1 to F-Actin by additionally generating an hBin1-SNAP variant, which can be labeled fluorescently. hBin1-SNAP binds F-actin in a co-sedimentation assay

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in a comparable manner to hBin1 (Fig EV1B). Interestingly, hBin1-SNAP readily associated with actin filaments and decorated the filaments like "pearls on a string" when analyzed by dual-color TIRFM (Fig 1D). hBin1-SNAP also induced actin bundling (Fig EV4B) and time-lapse analysis revealed that bundling events often occurred at hBin1-SNAP densely decorated actin filaments. However, whether hBin1 multimerization is a prerequisite for actin bundling needs to be tested in future studies.

Minor comments:

1. I think the evidence supporting the interaction between Bin1 and actin is compelling. But strictly speaking, I am not sure whether the author could conclude that Bin1 preferentially binds to F-actin. In Fig 1B, it looks like the relative ratio of Bin1 in solution vs. pellet is similar to the ratio of actin in solution vs. pellet. It will be great if the band intensity could be quantified. Similarly, Fig 1D could also be explained if Bin1 bind to actin in the monomeric form. The distinction may not be critical for the main conclusions of the paper but it will have important implications with regard to Bin1's binding site on the actin.

The reviewer raised an interesting question. It is true that we couldn't rule out a potential binding of Bin1 also to the monomeric form of actin although hBin1 had no effect on actin polymerization dynamics as shown in our pyrene assays in Fig 3A+B. Since we cannot discriminate in the co-sedimentation assay if hBin1 is interacting with monomeric actin in the supernatant fraction or if it is unbound, we addressed the G-actin binding experimentally. By using G-actin coupled sepharose beads we could pull down potential interactors via low-speed centrifugations. In this setup, hBin1 had an affinity for G-actin although it was weak compared to the G-actin binding protein profilin (Fig EV1A). Taken together, we think hBin1 can bind both forms, the monomeric and the filamentous form of actin, but so far we only observed Bin1-induced changes in actin dynamics when it bound to F-actin. These observations can be addressed in further detail in more structural studies, for which our mass spectrometry data may provide a suitable starting point.

2. BAR proteins tend to aggregate at low salt conditions, i.e. the conditions where the authors perform the actin polymerization assays. I am wondering whether this could affect Fig 2E/F.

The reviewer raises an important point here. But we observed that 50 mM KCl (the standard salt concentration suggested for every actin assay) does not have any influence on Bin1/BAR solubility. However, to further address this comment fully, we repeated this experiments with a dBAR-only control without F-actin. Here dBAR could not be found in the pellet fraction (Fig 2E+F), confirming the solubility of our purified recombinant proteins.

3. The fittings in Fig 3C do not visibly match with the original data.

Thank you for pointing out this apparent mismatch. We went back to our original data and found a small mistake in the data presentation from our exponential decay calculation. We used the values without prior subtracting the buffer-only value (our zero-values). We addressed this issue now and present the fittings associated to the original data in the refined Figure.

4. In light of findings in Fig 4A, are the EM image in Fig 1C in fact an actin bundle? If not, did the authors take a look at the ultrastructure of the EM bundles formed using the conditions of Fig 4A?

We repeated our EM studies under the same conditions of Fig 4A. Indeed, we could observe hBin1-induced actin bundling (Fig 4C). Remarkably, the bundles consisted of 2-5 filaments/bundle, which is comparable to the bundle analysis from our TIRFM data. We have added a descriptive section into the revised manuscript.

5. Page 10, line 10, should be "to some extent".

We thank the reviewer for pointing that out and changed the manuscript accordingly.

Referee #2

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Here the authors demonstrate that BIN1 can bind actin and alter actin dynamics in vitro and probably in vivo. While it is not terribly surprising that BIN1 binds actin through its BAR domain since BAR domains in other proteins are known to bind actin, demonstration of actin binding and in vitro investigation of the effects on actin polymerization and bundling are important results. Since BIN1 has been linked to Alzheimer's disease and previous efforts to investigate the potential disease role of BIN1 have mostly been to link BIN1 with Abeta through APP processing, a mechanistic focus on BIN1 and tau is welcome and likely to be of interest to a broad audience. The manuscript does not go very far in probing the in vivo interactions of BIN1, actin and tau; however, the focused nature of the manuscript fits with the scope of EMBO Reports.

A recent study in Developmental Cell (2015;35:186) suggests that the SH3 domain, not the BAR domain interacts with actin. The authors should comment.

We thank the reviewer for pointing out this important issue. Indeed, D'Alessandro et al. suggest a binding between Bin1 and actin via Bin1's SH3 domain. In order to test whether the SH3 domain might be also involved in F-actin binding, we additionally cloned and purified the SH3 domain of hBin1 (isoform 1) and subjected it to co-sedimentation assays with increasing F-actin concentrations. Under our experimental conditions, we could not observe any binding of hSH3 to F-actin (Fig EV2A+B). This discrepancy in the binding affinities could result from different protocols used (i.e. buffer conditions), the difference in isoforms and protein tags tested or the physicochemical properties of the generated recombinant proteins.

Referee #3

Summary:
This manuscript investigates the F-actin bundling properties of Bin1 (amphiphysin2). Bin1 has a N-terminal N-BAR domain and a C-terminal SH3 domain. The N-terminal domain binds to curved lipid membranes, while the SH3 domain has multiple interactions with other proteins, including some actin regulators.

The authors report that Bin1 binds weakly to F-actin, inducing the bundling of actin filaments. The authors provide results suggesting that such interaction occurs through the BAR domain. F-actin bundling by Bin1 provides a weak resistance to depolymerization in the presence of ADF/cofilin. This effect is conserved when actin bundles are simultaneously decorated by tau. As a consequence, the authors suggest that Bin1 could be an interesting target in taupathies, where an accumulation of actin rods in brain cells could be limited by a downregulation of Bin1.

This manuscript suffers from a number of problems listed below.

1/ D'Alessendro et al. (Dev Cell, 2015) already report the binding of Bin1 from C. elegans to F-actin. Therefore, the results from Figure 1 are not as novel as it is suggested.
We thank the reviewer for raising this important issue. Indeed, D'Alessandro et al. suggest a binding between Bin1 and F-actin in a co-sedimentation assay and we now directly address this finding in our revised manuscript. In particular, our findings in Figure 1 are based on several independent biochemical techniques, including EM data, dual-color TIRFM and a Kd calculation for the neuron-specific isoform 1 (D'Alessandro et al tested only the muscle-specific isoform 8), significantly expanding their initial findings and further suggesting that actin binding is a conserved function within the Bin1 family.

2/ The same study (D'Alessendro et al.) demonstrates clearly that the interaction between Bin1 and F-actin depends on its SH3 domain. In this current manuscript, using protein crosslinking and mass spectrometry analysis, the authors reach the opposite conclusion that the interaction is mediated through the BAR domain.
The authors need to explain why their result is different, and to provide data indicating the binding affinity of various Bin1 constructs to F-actin.
We had already gathered very broad evidence from our crosslinking, mass-spectrometry and the dBAR-only mutant, which suggested that the interaction between Bin1 and F-actin occurs at the BAR domain as already shown for the BAR domain proteins Pick1 and pacsin 2. In the revised form

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of the manuscript, we further characterize the binding abilities of hBAR to F-actin and find it binding similarly to full-length hBin1 (Fig EV2C+D). However, we cannot exclude that there are other, weaker actin binding sites next to the BAR domain as the main interaction site.

Therefore, we also tested the SH3 domain of hBin1 (isoform 1) in our co-sedimentation assay. The SH3 domain could not be found together with F-actin in the pellet fraction at any given actin concentration suggesting that it is not the main driver of the hBin1-actin interaction. This difference between both studies could be based on different experimental set-ups or that we tested different Bin1 isoforms. It is noteworthy that all our proteins (except hBin1-SNAP) have their protein tags cleaved off before being subjected to size exclusion chromatography and their use in the various biochemical assays. We would also like to note that we observed clear binding curves for hBin1 and hBAR on F-actin, while D'Alessendro et al. observe a rather linear increase in the amount of pelletable SH3 domain, that correlates directly with the amount of recombinant protein added into the experiment (see D'Alessendro et al. Figure 6), questioning a direct affinity relationship.

3/ Binding of BAR domain proteins to F-actin is often attributed to non-specific interactions between highly-charged membrane binding surfaces and a charged polymer. In the case of Bin1, a comparison of Figure 1D and Figure 2D indicates that the affinity of the BAR domain for F-actin is much weaker that the FL construct. Overall, the biological relevance of this interaction should be carefully questioned in vivo with specific point mutants to rule out any misinterpretation.
The questioned BAR domain we tested in Fig 2D was the BAR domain of the Drosophila ortholog of Bin1 (dBAR), which shares only 40% sequence identity with the human Bin1 (isoform 1) we tested in Fig 1D. When we compared the actin-binding ability of the full-length Drosophila Bin1 to its BAR domain in additional experiments, we observed comparable degrees of binding between the BAR domain and the full-length form (Fig EV1G).

In order to further address the reviewers concern, we additionally performed detailed binding studies using only the hBAR domain and compare that to our full-length hBin1 (Fig EV2C+D). hBAR also binds F-actin to a comparable degree than full-length hBin1. Moreover, we included hBAR in our pyrene depolymerization assays
where it could also stabilize actin filaments (Fig EV3) and hBAR induced actin bundling to a comparable extend (Fig EV4C) as observed via TIRFM. We have included these data and provide additional discussion in the revised manuscript.

The testing of BAR point mutations in detailed structural studies, which are based on our Mass-Spec results, are planned for future work, which we believe is out of scope for the current manuscript.

4/ Pyrene Assays are not convincing and misinterpreted. Why do actin assembly curves always drop for 20 min before showing a sudden increase?
I also disagree that the apparent increase in the rate of actin assembly in the presence of cofilin and Bin1 could be due to a stabilization of actin filaments against depolymerization. In a pyrene actin assembly assay, depolymerization is always negligible. An enhanced severing of actin filaments is more likely to explain this result.

We apologize that the reviewer misunderstood our representation of the pyrene assays. We realize that we didn't clearly state that the first minutes where the fluorescence signal was stable/dropped slightly was the G-actin without addition of the polymerization buffer. We recorded this signal as a "baseline", then paused the assay and added the polymerization buffer to induce polymerization. In order to make the figure easier to understand, we now excluded the baseline values and explain the graph in full detail. We agree with the reviewer that the increase in the rate of actin assembly in the presence of cofilin and Bin1 may suggest enhanced severing and addressed this point in our manuscript accordingly.
5/ Figure 6. The authors are lowering the expression of a full-length Bin1 construct. Given the fact that the SH3 domain of Bin1 interacts also with various actin regulators, it is difficult to conclude from this experiment that Bin1's bundling activity is what stabilizes tau bundles. The authors need to provide additional experiments confirming that BAR domain bundling activity is responsible of this observation.

We agree with the reviewer that our findings open up novel possibilities to test the role of Bin1 on tau pathology in more detail using in vivo models. These studies are planned. However, they are

currently beyond the scope of this manuscript. The generation of a new transgenic fly with a mutation/deletion in the BAR domain will clearly be part of more detailed future studies but cannot be generated in the given time frame for the revision. However, we strengthened the reviewer's point in our discussion section.

2nd Editorial Decision 02 August 2017

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see while referee 1 and 2 are positive about the study and support publication in EMBO reports, referee 3 is not convinced that the in vivo role of Bin1 in the fly brain has been sufficiently well documented. Upon further discussion the other referees agree that this reviewer is formally correct and that the in vivo evidence is not very strong. However, given that the main focus of the current manuscript is not so much on BIN1's role in AD but rather on its F-actin bundling properties, both referees suggested to address these concerns textually. Therefore, please discuss the limitations as outlined by referee 3 and potential other effects caused by BIN1 downregulation in the most appropriate manner in the manuscript. This could be along these lines: "... the in vivo phenotype is consistent with the mechanism proposed, but it does not exclude other possibilities such as..."

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

- Please remove the legend for Table EV1 from the paper and add it to the Dataset (in the first line of the Excel file). I suggest to submit this extensive table as Dataset instead of EV table. Please also change the callout in the manuscript accordingly.

- Please remove the legend for Movie EV1 from the manuscript. Provide the legend in an individual text file and then zip/compress this text file together with the movie.

- Please review the legend of figure 1 as the letters (a-g) do not match those in the figure panels.

- The Western blot shown in Fig. EV2A (middle panel) and in Fig. EV5C appear very over-contrasted. You might want to review these and provide a less strongly modified version.

We look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have questions or comments regarding the revision.

****

REFEREE REPORTS

Referee #1:

The authors have addressed all my concerns satisfactorily.
I therefore recommend its publication.

Referee #2:

The authors have appropriately responded to the issues raised by the referees.

Referee #3:

The authors provide a revised version where several experimental issues have been addressed. Overall, it is clearly established in this manuscript that Bin1's BAR domain interacts with actin filaments in vitro as many other BAR domains do. It is also established that this protein has a slight effect in slowing down actin depolymerization in vitro, which is something common for actin side-

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binding proteins.

Nevertheless, this manuscript is still problematic because the authors claim to provide experimental evidences for this effect in vivo. This is not the case as a downregulation of Bin1 in fly brains could have many other effects on the actin cytoskeleton than slowing down actin dynamics.

Minor comment:
Fig 3E: Curves have been swapped by accident.

2nd Revision - authors' response 07 August 2017

Referee #1:

The authors have addressed all my concerns satisfactorily.
I therefore recommend its publication.

Thank you.

Referee #2:

The authors have appropriately responded to the issues raised by the referees.

Thanks for the positive evaluation.

Referee #3:

The authors provide a revised version where several experimental issues have been addressed. Overall, it is clearly established in this manuscript that Bin1's BAR domain interacts with actin filaments in vitro as many other BAR domains do. It is also established that this protein has a slight effect in slowing down actin depolymerization in vitro, which is something common for actin side-binding proteins.

Nevertheless, this manuscript is still problematic because the authors claim to provide experimental evidences for this effect in vivo. This is not the case as a downregulation of Bin1 in fly brains could have many other effects on the actin cytoskeleton than slowing down actin dynamics.

Thank you for acknowledging the additional data provided in our revised version. We agree that downregulation of Bin1 in fly brains could modify actin dynamics by several mechanisms, not only by the proposed direct interaction. We addressed this issue now thoroughly in our discussion section as mentioned earlier.

Minor comment:
Fig 3E: Curves have been swapped by accident.

Thank you for pointing out this mistake. We changed the curve legends.

Accepted 08 August 2017

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Jahn, T.R.J
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2017-44137V1

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	All assays used in this study were pre-established from pilot experiments and planned acordingly.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No statistical method was used to determine sample sizes.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No Drosophila was excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	For scoring actin rods, pictures were blinded when scoring (Fig 6).
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	Mean-variance relationship was estimated empirically.
Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Information is provided in the material and methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	All details about Drosophila is provided in the materials and methods section.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No