THE EMBO JOURNAL

Manuscript EMBO-2016-95841

High capacity of the endoplasmic reticulum to prevent secretion and aggregation of amyloidogenic proteins

Lisa Vincenz-Donnelly, Hauke Holthusen, Roman Körner, Erik C. Hansen, Jenny Presto, Jan Johansson, Ritwick Sawarkar, F. Ulrich Hartl & Mark S. Hipp

Corresponding authors: F. Ulrich Hartl and Mark S. Hipp, Max Planck Institute for Biochemistry and Munich Cluster for Systems Neurology

Review timeline:

Submission date:	06 October 2016
Editorial Decision:	09 December 2016
Revision received:	01 June 2018
Editorial Decision:	23 July 2017
Revision received:	19 October 2017
Accepted:	25 October 2017

Editor: Anne Nielsen

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 09 December 2016

Thank you for submitting your manuscript to The EMBO Journal. Your manuscript has until now been handled by colleague Anne Nielsen, but as she is away from the office at the moment I am stepping in as secondary editor to avoid further delays. I would also like to apologize for the delay in getting back to you with a decision, but we have now received the needed input.

As you can see from the comments below, the manuscript received a bit of a mixed response. While referee #1 is not convinced that we get enough new insight, referees #2 and 3 are more supportive. However, it is clear that the analysis needs to be extended beyond resolving technical concerns. Referee #1 questions the biological relevance given that the findings are based upon using artificial proteins. I see this concern, but also see this study much more as a proof-of- concept study so I am not so worried about this issue. Where we need more insight is into the question why there is a difference between ER-beta and cytosolic-beta23in toxicity. Referee #2 suggests to look at Ca2+ levels. However there are probably other mechanisms as well that could be investigated.

So should you be able to address the concerns raised and add more insight into the difference in the handling of β-sheet proteins between the ER versus cytosol then we would be interested in considering a revised manuscript. I should add that it is EMBO Journal policy to allow only a single major round of revision and that it is therefor important to address the raised concerns at this stage. Maybe it would be good to discuss your outline for addressing the mechanism.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

Mark Hipp and colleagues present a set of nicely executed and well-documented data establishing that an artificial polypeptide rich in b-sheets and highly aggregation-prone in the cytosol reaches high concentration and remains soluble when expressed in the endoplasmic reticulum (ER).

This set of observations is in line with a previous study showing that the highly aggregation-prone mutant huntingtin reaches high concentration and is soluble in the ER. The biological relevance of the artificial proteins studied here is questionable. Moreover, as there is no insight into the mechanism accounting for the solubility of the ER-b protein, the reader is left to wonder about the significance of this study.

Should the authors wish to improve their manuscript, the following issues need attention:
1. The finding that the artificial b-peptide warrants cautious interpretation. It may lack proper signals to be efficiently secreted, this needs to be discussed.
2. It is surprising that the control ER-a protein is recovered in the media. The interpretation the authors provide is that the protein is secreted. More evidence to support this conclusion is needed. Can the author follow the trafficking of ER-a in the secretory pathway over time?
3. As mentioned above, the artificial protein may lack "proper" signal to be degraded by ERAD. This possibility needs to be highlighted.
4. Regarding the interactors of ER-b one wonders whether the interactions occur within the cells or post-lysis because some of the interactors recovered are from different subcellular compartments (mitochondria, nucleus). Controls are needed to distinguish between these two possibilities.
5. The authors found that ER-a is a better inducer of the UPR reporter than tunicamycin. This is very surprising. It will be useful to test other UPR inducers in this system to see if the observation still holds (for example Thapsigargin and DTT).
6. Fig 6C, D, E contain data both interesting and important but it is difficult to appreciate the effects because there is only one time points. The authors should perform more detailed time-course analyses similar to what they have done Fig2C.

Minor comments:
"Surprisingly" is used too often in the manuscript considering that the results are predictable.

Referee #2:

The authors have put an ER targeting signal on an artificial beta-sheet protein beta23 (ER-beta) and studied the impact that ER-beta lumen has on ER homeostasis. ER-beta originates from a library of artificial proteins designed to fold into 6 beta strands and is known to form amyloid-fibrils with cross beta structure in the human cytosol. As a control a similar construct was made with a model a-helical protein (ER-alpha). ER-alpha was secreted, whereas ER-beta was retained in the ER, but not degraded by ERAD. Yet, the toxicity of cytosolic beta-23 was diminished upon targeting it to the ER. To understand why ER-beta has reduced toxicity its oligomeric state was evaluated and data presented supports its assembly into a matrix like state.

IP/Mass spec studies show ER-beta interacts with a subset of ER chaperones that include BiP, Calnexin, SEL1, OS-9 and ERLIN-1. Interestingly, ER-beta sequestered around 50% of OS-9 and 10% of SEL1, yet ER-beta does not induce UPR. In contrast, ER-beta actually inhibited activity of a UPR reporter. An additional, toxic effect of ER-beta was the partial inhibition of the degradation of the ERAD substrate CPY*-mCH. Based on these data the authors discuss differences in the capacity of the ER and cytosol to manage accumulation on protein that are rich in beta structure.

The study is well organized and helps define compartment specific differences in mechanisms for management of toxic protein species. Yet, it is not entirely clear why ER-beta forms a matrix and cytosolic beta23 forms toxic assemblies?

The broad impact of the paper would have improved if this question was investigated from an additional angle. For example, Ca+ concentrations of the ER and cytosol are very different. Could it be that high Ca+ levels in the ER lumen impact the assembly of ER-beta into fibrils?

As is always the case with overexpression studies, there is some concern that the behavior of ER-beta are due to gross overexpression versus compartment specific matrix assembly. Does ER-beta form a matrix over a range of concentrations?

Sel1 overexpression limits the ability of ER-beta to inhibit ERAD of CPY*-mCH. At the same time, does overexpressed Sel1 alter the organization of ER-beta into detergent insoluble material or and immobile matrix?

Referee #3:

EMBOJ-2016-95841

The manuscript by Hipp, Hartl and coworkers entitled "High capacity of the endoplasmic reticulum to prevent secretion and aggregation of amyloidogenic proteins" is a very well-written and beautifully experimentally executed paper that should be seriously considered for publication in EMBO with only a couple suggested wording changes (very minor revisions-I do not need to see this paper again). This manuscript completes a very nice series wherein the same misfolding-prone beta-sheet rich protein is directed to different subcellular compartments. This manuscript shows that when the de novo designed beta-sandwich proteins that spontaneously aggregate into cross-beta-sheet or amyloid fibrils are directed to the Endoplasmic reticulum (ER) without an ER retention sequence, they accumulate their as soluble misfolded oligomers. Retention of soluble aggregates in the ER is much less cytotoxic than the accumulation of insoluble amyloid fibrils formed when these proteins reside in the cytosol (their previous paper). The ER-directed cross-beta-sheet soluble oligomers are recognized by the ER proteostasis network components and are retained in the ER by many factors, most prominently the Hsp70 Bip, without detectable secretion., as demonstrated by a series of experiments including unbiased proteomics. The accumulated ER-directed cross-beta-sheet soluble oligomers fail to induce the unfolded protein response, moreover these retained soluble aggregates inhibit thapsigargin from activating the UPR, which is a fascinating result that mirrors the inhibition of the heat shock reponse by the cytosolically directed cross-beta-sheet aggregates. The functional consequence of ER-directed cross-beta-sheet soluble oligomer accumulation is the sequestration of ERAD factors, thus these ER-directed aggregated beta-sheets inhibit the degradation of other ERAD clients, likely through SEL1L-a hypothesis supported by myriad of their experiements.

The main take-home of this paper, strongly supported by the author's data, is that the endoplasmic reticulum has a remarkable capacity to prevent the secretion of aggregation-prone proteins, while retaining soluble beta-sheet aggregates in a non-cytotoxic fashion with minimal detrimental effects on the cell, despite the fact that these cross beta-sheet aggregates do not readily get degraded, at least in the short term (long cell culture experiments are technically challenging). In contrast, accumulation of insoluble cross beta-sheet aggregates in the cytosol are dramatically cytotoxic by comparison.

It would be interesting to make a transgenic mouse ultimately to determine the long term effects of targeting cross beta-sheet aggregates to different cellular compartments, however this is well beyond the scope of this beautiful paper that should be published without delay.

Line 188 shouldn't "a dynamic network" become "oligomers" ? This sentence is confusing to me as

the photobleaching experiments show that these arregreates are not very dynamic.

1st Revision - authors' response 01 June 2018

EMBOJ-2016-95841 Response to Reviewers

Referee #1:

Mark Hipp and colleagues present a set of nicely executed and well-documented data establishing that an artificial polypeptide rich in b-sheets and highly aggregation-prone in the cytosol reaches high concentration and remains soluble when expressed in the endoplasmic reticulum (ER).

This set of observations is in line with a previous study showing that the highly aggregation-prone mutant huntingtin reaches high concentration and is soluble in the ER. The biological relevance of the artificial proteins studied here is questionable. Moreover, as there is no insight into the mechanism accounting for the solubility of the ER-b protein, the reader is left to wonder about the significance of this study.

Should the authors wish to improve their manuscript, the following issues need attention:
1. The finding that the artificial b-peptide warrants cautious interpretation. It may lack proper signals to be efficiently secreted, this needs to be discussed.
-We believe that the secretion competence of a protein containing an identical ER-targeting sequence (but lacking further posttranslational modifications) to be secreted, controls for this possibility (see 2). However we cannot rule out that the three aggregation-prone proteins that we studied may possess properties other than their aggregation tendency that prohibits them from leaving the ER. We have now added a sentence to the discussion that acknowledges this possibility.

2. It is surprising that the control ER-a protein is recovered in the media. The interpretation the authors provide is that the protein is secreted. More evidence to support this conclusion is needed. Can the author follow the trafficking of ER-a in the secretory pathway over time?
We clearly show that ER-alpha is present in conditioned medium. In addition, we show that BFA treatment reduces the levels of ER-alpha in the medium and that ER-alpha can also be detected inside the Golgi. Furthermore, we observe ER-alpha in vesicles that move rapidly through the cell (see file "Movie for Reviewer.avi"). Taken together, these findings are strong indicators for "classic" secretion of this protein. The fact that the medium is devoid of other intracellular proteins also suggests that ER-alpha is not present in the medium due to cell lysis that could serve as an alternative explanation for the appearance of non-secreted proteins outside of the cell.

3. As mentioned above, the artificial protein may lack "proper" signal to be degraded by ERAD. This possibility needs to be highlighted.
We have included a sentence in the discussion that acknowledges this possibility.

4. Regarding the interactors of ER-b one wonders whether the interactions occur within the cells or post-lysis because some of the interactors recovered are from different subcellular compartments (mitochondria, nucleus). Controls are needed to distinguish between these two possibilities.
We agree with the reviewer that interactions between ER-beta and proteins of other cellular compartments like mitochondria and the nucleus might be due to post-lysis interactions. We now state this in the text, and we have removed the panel that shows enrichment of mitochondrial proteins.

5. The authors found that ER-a is a better inducer of the UPR reporter than tunicamycin. This is very surprising. It will be useful to test other UPR inducers in this system to see if the observation still holds (for example Thapsigargin and DTT).
We have now also compared the effect of Thapsigargin and Tunicamycin with ER-alpha and see a comparable increase of the luciferase effects for all three conditions (please see attached image). However, we have now completely reorganized this section of the manuscript, to include RNA-seq data instead of luciferase based reporters.

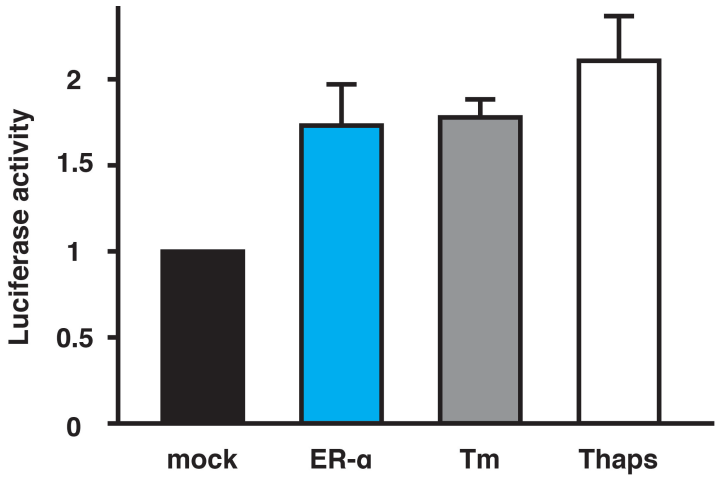

Figure for Referee #1 Point 5: ER-α, Tunicamycin and Thapsigargin lead to a comparable increase of UPRE controlled luciferase activity. HEK293T cells were co-transfected with a plasmid containing luciferase under control of the UPRE promoter and either empty pcDNA3.1 or ER-α in a ratio of 1:1. 24 h after transfection cells were split and seeded in 96-well plates in 100 µl DMEM. Another 24 h later, cells were treated with 1ug/mL tunicamycin or 1uM Thapsigargin. After additional 24 h 30 µl of Steady-Glo Luciferase Assay system buffer (Promega) were added directly to the wells followed by incubation for 15 min in the dark at room temperature. Luminescence was recorded in a plate reader (Clariostar, BMG Labtech) and did not differ significantly between ER-α and Tunicamycin (p=0.78) or Thapsigargin (p=0.15) treated cells. n=3, Error bars indicate SD.

6. Fig 6C, D, E contain data both interesting and important but it is difficult to appreciate the effects because there is only one time points. The authors should perform more detailed time-course analyses similar to what they have done Fig2C.
This figure is now redesigned, and we have now included a panel in Figure 5 that shows the stabilization of an ERAD substrate at additional time points.

Minor comments:
"Surprisingly" is used too often in the manuscript considering that the results are predictable.
We reduced the use of that term.

Referee #2:

The authors have put an ER targeting signal on an artificial beta-sheet protein beta23 (ER-beta) and studied the impact that ER-beta lumen has on ER homeostasis. ER-beta originates from a library of artificial proteins designed to fold into 6 beta strands and is known to form amyloid-fibrils with cross beta structure in the human cytosol. As a control a similar construct was made with a model a-helical protein (ER-alpha). ER-alpha was secreted, whereas ER-beta was retained in the ER, but not degraded by ERAD. Yet, the toxicity of cytosolic beta-23 was diminished upon targeting it to the ER. To understand why ER-beta has reduced toxicity its oligomeric state was evaluated and data presented supports its assembly into a matrix like state.

IP/Mass spec studies show ER-beta interacts with a subset of ER chaperones that include BiP, Calnexin, SEL1, OS-9 and ERLIN-1. Interestingly, ER-beta sequestered around 50% of OS-9 and 10% of SEL1, yet ER-beta does not induce UPR. In contrast, ER-beta actually inhibited activity of a UPR reporter. An additional, toxic effect of ER-beta was the partial inhibition of the degradation of the ERAD substrate CPY*-mCH. Based on these data the authors discuss differences in the capacity of the ER and cytosol to manage accumulation on protein that are rich in beta structure.

The study is well organized and helps define compartment specific differences in mechanisms for management of toxic protein species. Yet, it is not entirely clear why ER-beta forms a matrix and cytosolic beta23 forms toxic assemblies?

The broad impact of the paper would have improved if this question was investigated from an additional angle. For example, Ca+ concentrations of the ER and cytosol are very different. Could it be that high Ca+ levels in the ER lumen impact the assembly of ER-beta into fibrils?
To test the influence of calcium levels on the aggregation of ER-beta, we analyzed the mobility and detergent solubility of ER-beta in the presence of the SERCA inhibitor Thapsigargin, which depletes calcium in the ER, and have added new experiments that show that Thapsigargin is neither changing the observed detergent solubility of ER-beta nor its reduced mobility, which are indicative of ER-beta being present in a matrix-like state.

As is always the case with overexpression studies, there is some concern that the behavior of ER-beta are due to gross overexpression versus compartment specific matrix assembly. Does ER-beta form a matrix over a range of concentrations?
Thank you for this suggestion. We have now included an additional experiment that addresses the influence of ER-beta expression levels. To this end we compared the mobility of ER-beta-mCherry at different concentrations. Although we detected a small decrease of ER-beta mobility in cells expressing high levels of ER-beta, this difference was not statistically significant when compared with cells that expressed low levels (~20%) of ER-beta (new Supplemental Figure 5).
Sel1 overexpression limits the ability of ER-beta to inhibit ERAD of CPY*-mCH. At the same time, does overexpressed Sel1 alter the organization of ER-beta into detergent insoluble material or and immobile matrix?
We have now included a supplemental Figure that addresses this question (Supplemental Figure 6A). SEL1 overexpression leads to a slight increase of the mobility of ER-beta, however, this effect was very subtle and did not reach statistical significance.

Referee #3:

It would be interesting to make a transgenic mouse ultimately to determine the long term effects of targeting cross beta-sheet aggregates to different cellular compartments, however this is well beyond the scope of this beautiful paper that should be published without delay.
We agree that it would be fascinating to see the effects of ER-beta expressed in multicellular animals over a long time, but as the reviewer notes, this would be beyond the scope of this manuscript.

Line 188 shouldn't "a dynamic network" become "oligomers" ? This sentence is confusing to me as the photobleaching experiments show that these arregreates are not very dynamic.
We agree with this point and have removed the term dynamic in this sentence and also at other places were it is misleading

2nd Editorial Decision 23 July 2017

Thank you for submitting a revised version of your manuscript, it has now been seen by two of the original referees whose comments are shown below. As you will see, ref #2 is satisfied with the revision while ref #1 finds that the original concerns about biological and mechanistic advance remain unaddressed. Given these divergent views we have also consulted with an arbitrating advisor (who has seen the revised version of the study but not the referee reports from the previous round) and this person's comments are included below.

Given the overall positive recommendations from both ref #2 and our external advisor I would like to invite you to submit a final revision of the study in which you clarify/discuss the few minor points raised by our advisor as well as the following editorial issues concerning text and figures.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward receiving you final revision.

REFEREE REPORTS

Referee #1:

The biological relevance of the artificial proteins studied here remains questionable. There is no further insight in the mechanism accounting for the solubility of the ER-b protein. Last but not least, the authors highlight the technical limitations of their experiments and " agree with the reviewer that interactions between ER-beta and proteins of other cellular compartments like mitochondria and the nucleus might be due to post-lysis interactions."
This revised manuscript has not been significantly improved.

Referee #2:

The authors have responded to all of the concerns expressed in the previous round of review by including new experimental data or additional discussion. I believe that the data supports the major claims of the manuscript and provides new insights into mechanisms for handling protein aggregates in the ER.

Arbitrating expert advisor:

The revised manuscript from Hipp and colleagues significantly extends previous findings that the ER lumen is exceptionally capable of preventing the formation of aggregated proteins. Here, a previously developed pair of synthetic, cytosolic aggregating and non-aggregating peptides were targeted to the ER lumen, and their fates were examined. The data convincingly demonstrate that the ER-beta species is not secreted, fails to form TritonX-100 insoluble species, and unlike the ER-alpha (non-aggregating) protein ER-beta is retained within the ER. It is likely that a chaperone network binds ER-beta, yet the authors found that calcium depletion-which has been proposed to construct the gel-like features of the ER-had no effect on substrate mobility or detergent solubility. Furthermore, SILAC analysis suggested that ERAD inhibition due to the presence this stable, highly expressed aggregate arises from interference with the function of select ERAD components, most notably SEL1L. Impressively, "mild over-expression" of SEL1L was sufficient to rescue the ERAD defect. Finally, although one might have expected that ER-beta would induce a UPR based on these data, only a mild response was noted. However, this is consistent with other reports that ER oligomers similarly fail to elicit a UPR. Overall, this is an interesting report that required the completion of a series of technically challenging experiments. The biological relevance of this study lies in the explanation of numerous previously observed phenomena, and helps define which proteins in the ER might be most susceptible to the presence of toxic, aggregation-prone proteins.

Minor comments/questions:

Does ER-alpha induce a UPR? This might further validate the "control" for these experiments.

The authors should note that oligomers formed by the Z variant of alpha-1 antitrypsin (not only neuroserpin) in the ER also failed to induce a UPR. This should be referenced.

The citations for the percentage of the cell occupied by the ER (p. 7) should be updated to include recent work from Lippincott-Schwartz and colleagues (Nature).

The authors should comment on the migration of the ER-alpha species in the native gel (Fig. 4A). Does this arise from self-association, or chaperone binding?

The synthetic sequence used to construct ER-beta does not appear to contain any Cys residues. Based on pronounced binding to PDIA6, might this arise from a previously suggested chaperone-like activity of the isomerase?

In the Discussion (p. 16), mention is made of the TTR and light chain diseases, but in these cases the

proteins do pass ERQC but instead aggregate in the serum. Thus, the logic of linking the results in this study to these examples is not clear.

2nd Revision - authors' response 19 October 2017

Arbitrating expert advisor:

The revised manuscript from Hipp and colleagues significantly extends previous findings that the ER lumen is exceptionally capable of preventing the formation of aggregated proteins. Here, a previously developed pair of synthetic, cytosolic aggregating and non-aggregating peptides were targeted to the ER lumen, and their fates were examined. The data convincingly demonstrate that the ER-beta species is not secreted, fails to form TritonX-100 insoluble species, and unlike the ER-alpha (non-aggregating) protein ER-beta is retained within the ER. It is likely that a chaperone network binds ER-beta, yet the authors found that calcium depletion-which has been proposed to construct the gel-like features of the ER-had no effect on substrate mobility or detergent solubility. Furthermore, SILAC analysis suggested that ERAD inhibition due to the presence this stable, highly expressed aggregate arises from interference with the function of select ERAD components, most notably SEL1L. Impressively, "mild over-expression" of SEL1L was sufficient to rescue the ERAD defect. Finally, although one might have expected that ER-beta would induce a UPR based on these data, only a mild response was noted. However, this is consistent with other reports that ER oligomers similarly fail to elicit a UPR. Overall, this is an interesting report that required the completion of a series of technically challenging experiments. The biological relevance of this study lies in the explanation of numerous previously observed phenomena, and helps define which proteins in the ER might be most susceptible to the presence of toxic, aggregation-prone proteins.

Minor comments/questions:

Does ER-alpha induce a UPR? This might further validate the "control" for these experiments.
> Previous versions of this manuscript, and experiments included in the response to comments of referee#1 showed that ER-alpha induces the UPR, utilizing an UPR sensor that expresses firefly luciferase under control of the UPRE promoter.
We have however reorganized this section to include RNA-seq data instead of luciferase based reporters.
The authors should note that oligomers formed by the Z variant of alpha-1 antitrypsin (not only neuroserpin) in the ER also failed to induce a UPR. This should be referenced.
> We now mention this, and have added a reference (Hidvegi et al., JBC 2005) that states this fact
The citations for the percentage of the cell occupied by the ER (p. 7) should be updated to include recent work from Lippincott-Schwartz and colleagues (Nature).
> We have added this reference (Valm et al., Nature 2017), and modified the numbers accordingly.

The authors should comment on the migration of the ER-alpha species in the native gel (Fig. 4A). Does this arise from self-association, or chaperone binding?
> Since the antibody signal at higher molecular weights in the lane for ER-alpha is comparable to control cells transfected with pcDNA we interpret this signal as background. We have rephrased this section in the manuscript to make that point clearer.

The synthetic sequence used to construct ER-beta does not appear to contain any Cys residues. Based on pronounced binding to PDIA6, might this arise from a previously suggested chaperone-like activity of the isomerase?
> ER-beta contains a single cysteine so we cannot exclude a role of the isomerase activity. However our experiments with ER-beta-mCherry variants without this cysteine did not show any results that indicated a role of this residue. We have added a reference to emphasize the role of PDIA6 as a potential chaperone (Kikuchi et al., J.Biochem 2002)
In the Discussion (p. 16), mention is made of the TTR and light chain diseases, but in these cases the proteins do pass ERQC but instead aggregate in the serum. Thus, the logic of linking the results in this study to these examples is not clear.
> We have rephrased this passage, and removed the section connecting amyloidosis and failure of retention.

3rd Editorial Decision 25 October 2017

Thank your for submitting the revised version of your manuscript. I am pleased to inform you that your study has now been officially accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Mark S. Hipp
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2016-95841

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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?	For Western blotting around 300,000 cells were used per sample. For microscopy an average of 100 cells were visually inspected before taking around 10 representative images . For cell viability analysis 100,000 cells per sample were seeded out after transfection and analysed 72 h later. Each experiment was repeated at least three times (biological replicates) to ensure statistical significance. This experimental design is standard practice in the field.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded from the analysis.
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.	Cells were randomly allocated to different treatment groups.
For animal studies, include a statement about randomization even if no randomization was used.	NA
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.	Samples were labelled with numbers to minimize bias.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
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.	Yes
Is there an estimate of variation within each group of data?	Standard deviation was calculated as a measure of variation between biological replicates. Standard deviations are shown in the figures as error bars.
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com	Antibodypedia
http://1degreebio.org	1DegreeBio
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo	ARRIVE Guidelines
http://grants.nih.gov/grants/olaw/olaw.htm	NIH Guidelines in animal use
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm	MRC Guidelines on animal use
http://ClinicalTrials.gov	Clinical Trial registration
http://www.consort-statement.org	CONSORT Flow Diagram
http://www.consort-statement.org/checklists/view/32-consort/66-title	CONSORT Check List
http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tum	REMARK Reporting Guidelines (marker prognostic studies)
http://datadryad.org	Dryad
http://figshare.com	Figshare
http://www.ncbi.nlm.nih.gov/gap	dbGAP
http://www.ebi.ac.uk/ega	EGA
http://biomodels.net/	Biomodels Database
http://biomodels.net/miriam/	MIRIAM Guidelines
http://jji.biochem.sun.ac.za	JWS Online
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

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).	Primary antibodies used for immunoblotting: Anti-BiP/GRP78 (rabbit pAB, ab21685) Abcam, Cambridge, United Kingdom Anti-Calnexin (rabbit pAB, SPA860) Enzo Life Sciences Inc., Farmingdale, New York, USA Anti-Erlin-2/SPFH2 (rabbit mAB, ab128924) Abcam, Cambridge, United Kingdom Anti-GAPDH (mouse mAB, MAB374) Merck Millipore, Billerica, MA, USA Anti-GFP (mouse, mAB, 11814460001) Roche, Basel, Switzerland Anti-GRP94 (rat mAB, MA3-016) Thermo Fisher Scientific, Waltham, MA, USA Anti-HYOU1 (rabbit, mAB, ab134944) Abcam, Cambridge, United Kingdom Anti-mCherry (rat, mAB, M11217) Life Technologies, Carlsbad, CA, USA Anti-Myc (mouse mAB produced Max Planck Institute of Biochemistry, in hybridoma cell line Myc-9E10) Martinsried, Germany Anti-OS-9 (rabbit mAB, ab109510) Abcam, Cambridge, United Kingdom Anti-PDIA6 (rabbit mAB, ab154820) Abcam, Cambridge, United Kingdom Anti-SEL1L (rabbit pAB, S3699) Sigma Aldrich, St. Louis, MO, USA Anti-α-Tubulin (mouse mAB, T5168) Sigma Aldrich, St. Louis, MO, USA Secondary antibodies used for immunoblotting Anti-mouse IgG-Peroxidase (goat pAB, A4416) Sigma Aldrich, St. Louis, MO, USA Anti-rat (goat pAB, A9037) Sigma Aldrich, St. Louis, MO, USA Anti-rabbit (goat pAB, A9169) Sigma Aldrich, St. Louis, MO, USA Primary antibodies used for immunofluorescence: Anti-Calreticulin (chicken pAB, ab14234) Abcam, Cambridge, United Kingdom Anti-ERp57 (rabbit pAB, ab10287) Abcam, Cambridge, United Kingdom Anti-Giantin (rabbit pAB, ab24586) Abcam, Cambridge, United Kingdom Anti-Myc (mouse mAB, sc-40) Santa Cruz Biotechnology, Dallas, TX, USA Secondary antibodies used for immunofluorescence: Anti-mouse Cy3 (goat pAB, 115-165-062) Jackson ImmunoResearch Laboratories, West Grove, PA, USA Anti-rabbit FITC (goat pAB, F2765) Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA Anti-rabbit Alexa Fluor 405 (goat pAB, A-31556) Life Technologies, Carlsbad, CA, USA Anti-chicken Alexa Fluor 488 (goat pAB, A-11039) Life Technologies, Carlsbad, CA, USA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All cell lines were newly purchased from American Type Culture Collection (ATCC)/LGC Standards. Cell lines were regularly tested for mycoplasma contamination using PCR.

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

D- Animal Models

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.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
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.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
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.	NA

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	All sequencing data generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE98580
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).	NA
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).	NA
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.	NA

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