nature research

Peer Review Information

Journal: Nature Structural and Molecular Biology
Manuscript Title: BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1
Corresponding author name(s): Dr Rachel Klevit

Reviewer Comments & Decisions:

Decision Letter, initial version:

23rd Sep 2020

Dear Dr. Klevit,

Thank you again for submitting your manuscript "BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1". We have now received the comments (copied below) from the 3 reviewers who evaluated your paper. In light of their reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

I hope you will be pleased to see that all 3 reviewers are quite positive about the work, and that each offers specific suggestions that we also agree would improve its presentation. Reviewer #1, with expertise in BRCA1/BARD1 function, notes that the BRCA1-E2 fusion construct isn't sufficiently described, and also suggests presenting an overlay with RING:E2 structures to show how the Ub moiety is positioned. Reviewer #2, with cryoEM and NMR expertise, points to some overstatements that need modification, and requests an SDS-PAGE gel showing the purified, full-length BRCA1/BARD1 complex. Reviewer #3, with expertise in histone Ub'n, queries aspects of the TROSY data, and also requests an additional assay confirming that the full-length BRCA1/BARD1 complex displays the same specificity as the RING-domain construct to reinforce the biological relevance of the findings.

Please be sure to address/respond to all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file. If you have comments that are intended for editors only, please include those in a separate cover letter.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome. Please feel free to contact me directly if you have any concerns about the feasibility of the reviewers' requests.

To facilitate revising the manuscript, I am providing some guidelines for our Article format:

- abstract should be under 150 words, no references;
- main text is typically between 3,000 and 4,000 words, and should be organized as introduction, results (with subheadings) and discussion.
- display items (figures and tables): typically between 6 and 8. Please note that the cryoEM data table should be in main article.
- supplementary items: There should be max. 10 Supplementary Figures; other allowed supplementary items are Suppl Table, Note, Video, Data Set.
- uncropped images of gels and blots should be presented in a Supplementary Data Set.

Please note that we might need to consult at least a subset of the original reviewers, so do prepare a point-by-point response that can be sent to them.

We expect to see your revised manuscript within 6 weeks. If you cannot send it within this time, please contact us to discuss an extension; we would still consider your revision, provided that no similar work has been accepted for publication at NSMB or published elsewhere.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf

Please note that the form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labeled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the production process or after publication if any issues arise.

FOR MS WITH CROPPED GELS: Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. These data can be aggregated into a single supplementary figure item. While these data can be displayed in a relatively

informal style, they must refer back to the relevant figures. These data should be submitted with the final revision, as source data, prior to acceptance, but you may want to start putting it together at this point.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

Nature Structural & Molecular Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Once they are ready, please use the link below to submit your revised manuscript and related files:

[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

With kind regards,

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
This study describes the comparison of BRCA1-BARD1 with BMI1-RING1B E2 interactions with the H2A and nucleosome. The findings are highly insightful, describing a unique role for BARD1 in 'tipping' the E2 away from the canonical H2A modificaion site, K118/9. Further, the finding that the H2A C-terminal tail structure differs between the two E3-E2 structures is an important insight.

The study is original and important, giving further evidence of the modification of C-terminal H2A by BRCA1-BARD1, providing vital information as to how this specificity is achieved, an pointing to evidence that the E3 function – in particular in reference to histone modification- may be important in cancer. The study is well written, beautifully presented and accessible.

The structure has been made possible by cross-linking and by fusing the E3 genetically to the E2 using a linker sequence. While this is somewhat contrived I think we have to live with it as a solution to a difficult problem. However, the approach not fully described. No actual description of what the link between BRCA1 and the E2 is given (no sequence). Thus the community can't recapitulate the findings. Perhaps this is a result of a surfeit of precaution while the manuscript is in review, it is unacceptable for publication.

It is a pity that no ubiquitin is in the structure (although I accept it isn't possible using a fused E2). Nevertheless, it would be helpful to include an overlay with RING: ubiquitin-E2 structures, at least in the discussion to give the reader an idea of where the ubiquitin is expected to be, and whether it might be predicted to contribute to positioning, (rather than not shown).

The authors note that Ubiquitiantion of methylated nucleosomes (H3 K79) is poor. However, to say this is consistent with BRCA1 being transcriptional repressive is an overstatement. It suggests that BRCA1:BARD1 ligase function may occur more frequently at non-transcribed regions.

Overall in my view this is an excellent, important study.

Reviewer #2:
Remarks to the Author:

The authors describe a substantial set of experiments that reveal how the RING-heterodimer of human BRCA1/BARD1 (fused to UbcH5c) binds to and recognises a nucleosome particle (by cryoEM). They then validate and confirm the observed mode of binding through a set of biochemical and biophysical experiments and differentiate it from that previously observed for the structurally similar Ring1b/Bm1 RING-heterodimer (from Polycomb repressor complex 1). They go onto show that ubiquitylation by BRCA1/BARD1-UbcH5c is sensitive to the methylation status of H3K79 and is also affected by mutations found in patients with certain types of cancer. They also propose a model that links the flexibility of the H2A C-terminal tail to the ability to ubiquitylate defined lysine residues, thus providing a rationale for how specificity may be achieved in different RING/substrate complexes.

Overall the manuscript is of a high quality with the results serving to support each of the authors' observations and hypotheses, and therefore warrants acceptance for publication.

On a more minor note, the manuscript does suffer in several places (in terms of readability) by the constant need to flip to / and from the supplementary material in order to fully follow their experimental process and ultimately their conclusions.

Major points:

Page 9. "The biochemical and structural properties of full-length human BRCA1/BARD1 have remained largely enigmatic due to challenges with isolation of high-quality recombinant protein"

This statement should be removed, as the authors cite Zhao W. et al, Nature 2017, which clearly shows that the previous issue in making the full-length proteins has now been resolved.

Page 9. Using highly purified full-length human BRCA1/BARD1 and an unmodified nucleosome substrate, we observe faster nucleosome ubiquitylation kinetics and a greater than 100-fold increase in nucleosome binding affinity compared to the RING heterodimer (FIGURE 4B-C)".

You cannot state that you have highly-purified full-length human BRCA1/BARD1 without showing a representative sample on an SDS-PAGE gel.

Minor points:

Figure 1D and 1E should be moved elsewhere, as they are not referred to in order by the manuscript's narrative.

All "error" values should be declared as either 1 standard deviation (1 SD) or as standard error of the mean (SEM) as appropriate; the use or 'error' or 'standard deviation' is not sufficiently specific.

Please clearly label *all* amino acids that shown in stick representation (e.g. see supplementary figure S5A, B, C).

Reviewer #3:
Remarks to the Author:
In metazoans, histone H2A is mono-ubiquitinated at multiple sites. The site of ubiquitin attachment determines the functional outcome, including transcriptional repression (at H2AK118/K119), DNA DSB repair (at H2AK13/15), and possibly both silencing and DDR (at H2AK127/K129). How the cellular machinery selectively read, write and erase these different signals is of particular interest. The manuscript by Witus et al. provides the structural and biochemical basis for how the BRCA1/BARD1 E3 ligase selectively ubiquitinates nucleosomes in the C-terminal tail of H2A. This work not only addresses the major mystery of how the specificity of BRCA1/BARD1 is distinct from RING1B/BMI1, but also brings us closer to fully understand the functional consequences of BRCA1/BARD1 mutations

that are associated with breast and ovarian cancer.

The cryoEM structure of a truncated version of BRCA1/BARD1/UbcH5 in complex with the nucleosome confirmed that the E3 ligase is anchored at the nucleosome acidic patch, as previously suggested. Surprisingly, the structure also revealed that subtle differences between how BARD1 and BMI1 interact with the nucleosome acts to pivot the E2 away from the nucleosome surface. Critically, this structural feature separates the E2 active site from the K118/119 sites targeted by RING1B/BMI1 by 19Å. It also prevents the E2 from interacting with nucleosomal DNA, thus lowering the affinity of the enzyme complex for its substrate relative to RING1B/BMI1/UbcH5. The authors presented a very thorough biochemical examination of the interface residues observed in the structure and the results strongly support the functional significance of the structure-based predictions. Additionally, by systematically installing single lysine in the H2A C-tail, the authors determined that the site specificity of ubiquitination is largely determined by accessibility of the lysine residue to the E2 active site. Overall, the data are of high quality and the experiments are rigorous. The length of the manuscript is appropriate. These results will elicit broad interests in multiple scientific communities.

I have two main suggestions for the authors:
1. The observations presented in Figure 6 are a bit puzzling. First, since RING1B/BMI1/UbcH5 binds to the NCP with much higher affinity, it's important to know that the concentrations used in the TROSY experiments are saturating for the BRCA1/BARD1/UbcH5 complex. To do this, a complete titration isn't needed; rather, showing that peak-broadening has plateaued at two E3 concentrations (e.g, concentrations that differ by 2-fold) would be sufficient. Second, the TROSY data suggest there is little interaction between the C-tail and the E2/E3 enzyme. The authors should comment on how common this is among other ubiquitination enzyme-substrate pairs and its implication in ubiquitination site selectivity in general.
2. Figure 7 suggests that full-length BRCA1/BARD1 is a much more efficient enzyme. Because this manuscript focuses on the question of specificity, it would be appropriate to show whether or not full-length BRCA1/BARD1 has a similar specificity profile as the truncated version; this experiment can use the set of single-lysine substrates as in Figure 5C. Without this comparison, it will be very difficult to gauge the physiological relevance of many of the results obtained with the truncated BRCA1/BARD1 complex.

Author Rebuttal to Initial comments

We are very pleased to read the overall positive response from reviewers. We thank them for their suggestions and hope that we have adequately addressed the concerns here and in the manuscript.

Reviewer #1:

No actual description of what the link between BRCA1 and the E2 is given (no sequence). Thus the community can't recapitulate the findings. Perhaps this is a result of a surfeit of precaution while the manuscript is in review, it is unacceptable for publication.

We apologize for this unintentional oversight. The exact residue bounds of the BRCA1UbcH5c chimera including the sequence of the GS-linker can now be found in the first paragraph of the methods section and are also in the PDB file to be released with this manuscript.

It is a pity that no ubiquitin is in the structure (although I accept it isn't possible using a fused E2). Nevertheless, it would be helpful to include an overlay with RING: ubiquitin-E2 structures, at least in the discussion to give the reader an idea of where the ubiquitin is expected to be, and whether it might be predicted to contribute to positioning, (rather than not shown).

We have included an additional supplemental figure (S10) that shows a structural alignment of our complex with three closed conformation E3/E2-Ub structures aligned to the BRCA1 RING domain. The alignment shows that Ub in a more closed conformation does not clash with the NCP.

The authors note that Ubiquitination of methylated nucleosomes (H3 K79) is poor. However, to say this is consistent with BRCA1 being transcriptional repressive is an overstatement. It suggests that BRCA1:BARD1 ligase function may occur more frequently at non-transcribed regions.

We agree and have updated the main text to reflect this.

Reviewer #2:
Remarks to the Author:

Major points:

Page 9. "The biochemical and structural properties of full-length human BRCA1/BARD1 have remained largely enigmatic due to challenges with isolation of high-quality recombinant protein"

This statement should be removed, as the authors cite Zhao W. et al, Nature 2017, which clearly shows that the previous issue in making the full-length proteins has now been resolved.

Thank you for pointing out this contradiction. We have removed this statement from the manuscript.

Page 9. Using highly purified full-length human BRCA1/BARD1 and an unmodified nucleosome substrate, we observe faster nucleosome ubiquitylation kinetics and a greater than 100-fold increase in nucleosome binding affinity compared to the RING heterodimer (FIGURE 4B-C)".

You cannot state that you have highly-purified full-length human BRCA1/BARD1 without showing a representative sample on an SDS-PAGE gel.

A Coomassie stained SDS-PAGE gel of purified FL-BRCA1/FL-BARD1 is now included in figure S9a.

Minor points:

Figure 1D and 1E should be moved elsewhere, as they are not referred to in order by the manuscript's narrative.

Figure 1D and 1E are presented in order in the second to last paragraph of the introduction. References back to figure 1C and 1D are also present in the subsequent results section.

All "error" values should be declared as either 1 standard deviation (1 SD) or as standard error of the mean (SEM) as appropriate; the use or 'error' or 'standard deviation' is not sufficiently specific.

All error values reported in the text correspond to 1 standard deviation (1-SD). We revised figure legend text to make this clear.

Please clearly label *all* amino acids that shown in stick representation (e.g. see supplementary figure S5A, B, C).

All amino acids that are portrayed as sticks are now labelled throughout all figures.

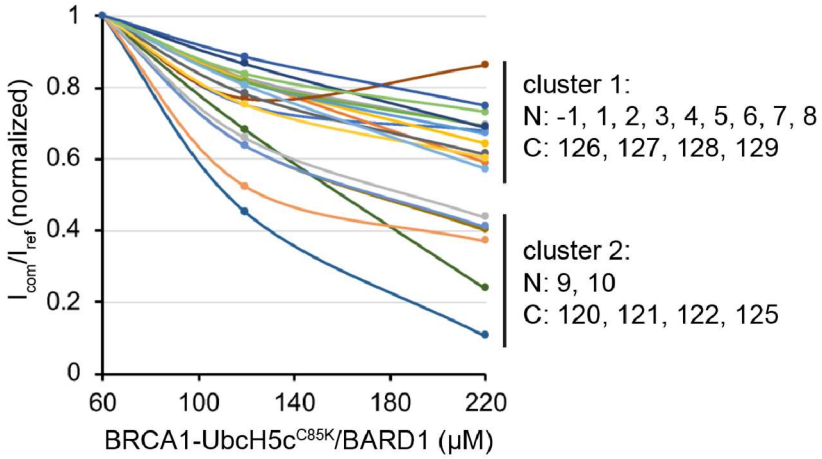
Reviewer #3:

I have two main suggestions for the authors:
1. The observations presented in Figure 6 are a bit puzzling. First, since RING1B/BMI1/UbcH5 binds to the NCP with much higher affinity, it's important to know that the concentrations used in the TROSY experiments are saturating for the BRCA1/BARD1/UbcH5 complex. To do this, a complete titration isn't needed; rather, showing that peak-broadening has plateaued at two E3 concentrations (e.g, concentrations that differ by 2-fold) would be sufficient. Second, the TROSY data suggest there is little interaction between the C-tail and the E2/E3 enzyme. The authors should comment on how common this is among other ubiquitination enzyme-substrate pairs and its implication in ubiquitination site selectivity in general.

Thank you for suggesting this experiment aimed at showing convincingly that both complexes are saturated at the concentrations in our NMR experiments. We initially used excess BRCA1-UbcH5c^{C85K}/BARD1 in our assays for this exact reason. While we are confident that the Ring1b-UbcH5c/Bmi1/nucleosome complex is saturated as the NMR concentrations are ~100-fold higher than the reported Kd and we observe visible aggregation after adding saturating amounts of E3-E2, we

cannot be as certain about the BRCA1-UbcH5c^{C85K}/BARD1/nucleosome complex. Unfortunately, we were unable to measure an accurate Kd for this complex by ITC. Nevertheless, our SEC-MALS data (Figure S2f) indicates that the complex co-elutes at the molecular weight expected for a 2:1 complex and is fairly mono-disperse (as judged by flat MALS line in the profile).

To try to better address the question raised, we performed a titration experiment in which increasing amounts of the BRCA1-UbcH5c^{C85K}/BARD1 E3-E2 complex were added (60, 120, 220 uM; figure S8c). We observed broadening of resonances corresponding to signals from both the N- and C- terminal tails of H2A at all titration points. At 220 μM E3E2, broadening of the N-terminal tail of H2A far surpassed values observed in the saturated Ring1b-UbcH5/Bmi1/nucleosome complex. We interpret this extra signal loss as coming from non-specific binding and supersaturation of the complex at the high NMR concentrations and low ionic strength required to observe these complexes. To gain insight into the behavior of individual residues in this experiment, we quantified the signal broadening for each resonance at the three titration points ($I_{complex}/I_{ref}$) and normalized these values to the 60 μM addition (below & figure S8d). This analysis reflects the degree to which various resonances broaden as a function of added binding partner. A majority of signals exhibit similar behavior (cluster 1), with some outliers (cluster 2). The outliers all arise from residues closest to the ordered regions of the histone, some of which are broadened beyond detection at high concentrations of E3E2 added. The resonances in cluster 1 belong to residues -1-8 from the N-terminal tail and 126-129 in the extreme C-terminal tail of H2A. This analysis implies that residues from the N-terminal tail of H2A and those of greatest interest from the C-terminal tail of
H2A (126-129) experience general broadening effects as a result of large excesses of E3-E2 in the experiment and there is no additional *specific* signal loss that we are missing in the extreme C-terminal tail of H2A as a result of the complex being undersaturated.



While we believe that the sum of our data (titration presented above, SEC-MALS, and similar broadening of H3 N-terminal tail between Ring1b/Bmi1 and BRCA1/BARD1 E3-E2 complexes (Fig S8g-h)) indicates that the BRCA1-UbcH5c^{C85K}/BARD1/nucleosome complex is saturated at the concentration used in our experiments, we cannot formally rule out undersaturation from the experiment described to fully address this reviewer concern. For this reason, we have altered our analysis to compare the broadening of the C-terminal tail to the N-terminal tails of H2A in each E3-E2/nucleosome complex and have removed wording and analysis comparing the degree of difference in broadening between the two E3-E2/nucleosome complexes (Figure 6b-c). This analysis, along with supplementary experiments (H3 tail monitoring and SEC-MALS) and those performed to address reviewer comments, clearly shows that the extreme C-terminal tail of H2A where Lys targets 127/129 reside retains similar flexibility to the N-terminal region of H2A and are not specifically conformationally restricted by formation of the E3E2/nucleosome complex. However, the same analysis shows that the C-terminal region of H2A is affected more than the N-terminal region in the Ring1b-
UbcH5c/Bmi1/nucleosome complex. We hope that our revised analysis and presentation adequately addresses the reviewer's concerns.

To address the second comment as to how common this mechanism is, we are unaware of any other study that has rigorously tested the effects of E3-E2 binding on the dynamics of a flexible region of a substrate that is specifically ubiquitylated.
Crystallographic studies have targeted RING/substrate pairs that ubiquitylate ordered lysine residues or use chemical methods to crosslink and capture E3/E2/substrate intermediates. We have commented on this in the discussion section and cited several key papers that have investigated RING E3/E2/substrate interactions.

2. Figure 7 suggests that full-length BRCA1/BARD1 is a much more efficient enzyme. Because this manuscript focuses on the question of specificity, it would be appropriate to show whether or not full-length BRCA1/BARD1 has a similar specificity profile as the truncated version; this experiment can use the set of single-lysine substrates as in Figure 5C. Without this comparison, it will be very difficult to gauge the physiological relevance of many of the results obtained with the truncated BRCA1/BARD1 complex.

We have included an assay showing the H2A lysine specificity of full-length (FL)- BRCA1/BARD1 by comparing its activity using a wild-type H2A nucleosome substrate to H2A Lys125/127/129Arg (3KR) nucleosome substrate (Figure S9b). These data clearly show a preference for Lys 125/127/129 in H2A, although there is some ubiquitylation of other lysine residues under our experimental conditions. We note that the H2A specificity profile of full-length BRCA1/BARD1 has been previously reported *in vitro* by single and combinatorial lysine mutation of lysine residues 118, 119, 125, 127, and 129 (Kalb *et al*. 2014. Cell Rep; Figure 3B). Using FL-BRCA1/FL-BARD1, these authors showed that only simultaneous

mutation of Lys 125/127/129, but not Lys 118/119, depleted H2A ubiquitylation activity. The authors of that study also showed that BRCA1/BARD1 specifically targets Lys 125/127/129 in cells (Kalb *et al*. 2014. Cell Rep; Figures 3D-E; S3G).

Decision Letter, first revision:

18th Nov 2020

Dear Dr Klevit,

Thank you again for submitting your manuscript "BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1". The reports of the referees are below, and based on these comments, we are happy to accept your paper, in principle, for publication as an Article in Nature Structural & Molecular Biology, on the condition that you revise your manuscript in response to the comments of the referees and our editorial requirements.

I hope that you will be pleased to see that the reviewers find that the revisions fully address their prior concerns.

The text and figures will require revisions. Note that, within a few days, we will send you detailed instructions for the final revision, along with information on editorial and formatting requirements. We recommend that you do not start revising the manuscript until you receive this additional information.

****To facilitate our work at this stage, we would appreciate if you could send us the main text as a word file. Please make sure to copy the NSMB account (cc'ed above).*****

Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

TRANSPARENT PEER REVIEW
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Please note: we allow redactions to authors' rebuttal and reviewer comments in the interest of confidentiality. If you are concerned about the release of confidential data, please let us know

specifically what information you would like to have removed. Please note that we cannot incorporate redactions for any other reasons. Reviewer names will be published in the peer review files if the reviewer signed the comments to authors, or if reviewers explicitly agree to release their name. For more information, please refer to our FAQ page.

ORCID

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https://www.springernature.com/gp/researchers/orcid/orcid-for-nature-research

IMPORTANT: All authors identified as 'corresponding author' on the manuscript must follow these instructions. Non-corresponding authors do not have to link their ORCIDs but are encouraged to do so. Please note that it will not be possible to add/modify ORCIDs at proof. Thus, if they wish to have their ORCID added to the paper they must also follow the above procedure prior to acceptance.

To support ORCID's aims, we only allow a single ORCID identifier to be attached to one account. If you have any issues attaching an ORCID identifier to your MTS account, please contact the Platform Support Helpdesk.

We hope that you will support this initiative and supply the required information. Should you have any query or comments, please do not hesitate to contact me.

In recognition of the time and expertise our reviewers provide to Nature Structural & Molecular Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1". For those reviewers who give their assent, we will be publishing their names alongside the published article.

If you have any questions, please do not hesitate to contact me directly.

With kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology

Reviewer #1 (Remarks to the Author):

The authors have improved the manuscript in each of the areas I requested.

I hope to see it published soon.

Reviewer #3 (Remarks to the Author):

The authors have fully addressed my comments. Thank you!

Author Rebuttal, first revision:

POLICY ISSUES:

1. Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as supplementary information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory.

Statistical data in graphs, NMR intensities, and uncropped blots/gels from all figures are included as source data.

2. DATA DEPOSITION: EM maps must be deposited in the EMDB. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. Accession codes must be provided in your final submission for acceptance, and entries must be accessible or HPUB at the galley proof stage.

EM maps, atomic coordinates, and NMR chemical shifts have all been deposited to the relevant databases and will be released with this paper. Accession codes are indicated.

3. Nature Research is taking an active approach to improving our transparency standards and increasing the reproducibility of all of our published results. Detailed information on experimental design and reagents is now collected on our Life Sciences Reporting Summary, which will be published alongside your paper. Please provide an updated version of the Reporting Summary (which will be published with the paper) with your final files.

An updated reporting summary is attached with the submission.

https://www.nature.com/authors/policies/ReportingSummary.pdf

Please also upload a revised Editorial policy checklist. https://www.nature.com/authors/policies/Policy.pdf

GENERAL FORMATTING:

4. The manuscript is currently 4321 words (main text; Introduction, 602 words; Results: 2832 words; Discussion: 887 words). Our standard word limit is 4,000 words for main text; in this case, we can permit 4300 words, but please do not exceed this limit during manuscript revision.

Our revised text including figure references is 4298 words.

5. Current title: BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1 (**10 words, 81 characters**, spaces included). [OK]

6. Your abstract is currently 150 words. It should remain max. 150 words, but should also define species of origin of the system being studied.

We have included human as the species of origin for the system in the abstract (149 words).

7. The Online methods section is currently 4116 words. We do not have a strict limit for this section but we suggest 3000 words as a target. I suggest presenting the cryoEM sample preparation, image processing, and model building sections together as a Supplementary Note.

We have moved all cryo-EM related methods to a Supplementary Note and are now well under 3000 words.

8. References: the current manuscript has 66 references in main text and 34 in methods. Up to 60 references are allowed in the main text; additional 20 references can be included in the online Methods. Please make sure all references are cited in numerical order and place Methods-only references

after the Methods section, following the numbering of the main reference list (**i.e. do not start at 1**).

We have updated our references to adhere to these guidelines.

9. References: the reference list should contain papers that have been published or accepted by a named publication or recognized preprint server. Published conference abstracts, numbered patents and research datasets that have been assigned a digital object identifier may also be included in the reference list.

All references are published journal articles or pre-prints.

10. Please avoid using slashes as in [BRCA1/BARD1; Ring1b/Bmi1]. Slashes are OK in ratios and units, genotypes, sequence motifs, and cotransport. Replace with parentheses (e.g., for homologs and alternate names), "and," "or," or hyphen as applicable; use en-dashes to separate components of a complex.

Thank you for your flexibility on this stylistic point (as previously discussed) to allow us to maintain clarity of the system being studied. .

FIGURES AND TABLES:

11. There are currently 7 Figures and 1 Table in main article. [OK] Tables should be pasted into Word files as editable tables, not as images.

The table has been pasted into word using the NSMB cryo-EM template.

12. Please make sure all figures and tables, including Extended Data Figures, are cited in the text in numerical order.

All main figures are cited in order. Extended Data figures 5, 6, and 7 are initially presented in numerical order, but sometimes re-reference out of order. This is due to the thematic rather than strictly sequential nature of content in these E.D. figs.

13. To streamline the production process, we have created templates for the structural statistics tables (attached). Please revise the table according to these templates; add rows for additional parameters if necessary.

The table has been pasted into word using the NSMB cryo-EM template.

14. Cropping of gel and/or blot images: gel pieces should be separated with white space (do not add borders). When cropped gels or blots are shown in the main figures, all key data should be presented in uncropped form with molecular weight markers, as Source Data, as instructed below. These data can be displayed in a relatively informal style, but must refer back to the relevant figures; figure legend text should refer to the uncropped image and cite the Source Data (e.g., Uncropped blot/gel images are shown in the Source Data").

All uncropped gels/blots are included as source data and referenced in figure legends. Borders have been removed from all gel images in main and E.D. figures.

15. *When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorialpolicies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:*

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading or sample processing controls
-- that all images in the paper are checked for duplication of panels and for splicing of gel lanes. Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the production process or after publication if any issues arise.

These integrity guidelines have been closely adhered to.

SUPPLEMENTARY INFORMATION

All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

1. EXTENDED DATA FIGURES: Extended Data Figures are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data Figures, and each must be referred to in the main text. Each Extended Data Figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends

must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.
All Extended Data Figure must be called out in order as Extended Data Fig. 1, Extended Data Fig 2, etc.

Extended data figures 1-10 have been uploaded as .jpg files, and all are under 10MB. Extended data figures 5, 6, and 8 contain too many panels to allow for the figure legend to be placed on the same page while retaining figure clarity. We request that you allow the figure legends corresponding to these 3 figures to be placed on the next page. Please see point #12 about out of order reference of ED figures in the text.

2. SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, large figures, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information.
Supplementary Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.
The cryo-EM methods will be the only SI included with this paper.

Supplementary items (such as Supplementary Tables, Videos, Notes, and additional Supplementary
Figures if permitted), should be numbered and called out in main article, as Supplementary Figure 1 (not SI1) and so on.

3. SOURCE DATA: We encourage you to provide source data for your figures. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Statistical source data (i.e., data behind graphs) should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (https://figshare.com/) or the Image Data Resource (https://idr.openmicroscopy.org).

Source data should be cited in the legend text (e.g., "Uncropped images for panels a-c are available as source data" or "Data for graphs in d-f are available as source data").

Source data for all graphs and uncropped gels/blots are included as separate excel files and pdfs for each main text figure and E.D. figure where relevant.

STATISTICS and
REPRODUCIBILITY

16. **[Bar graphs: e.g figures 3, 5, 6, S5, S6, S7, S8]** GRAPHS: we encourage replacing bar graphs with a dot-plot format, particularly for sample sizes of n<10, or to a box-and-whisker format to show data distribution.

We have considered this and decided to keep the graphs as they are. We note that all individual data points are included in the source data files.

17. GRAPHS: wherever statistics have been derived (e.g. error bars, box plots, statistical significance), the legend needs to provide and define the n number (i.e. the sample size used to derive statistics) as a precise value (not a range); the type of repeat should also be specified, using the wording "n=X biologically independent samples/cell cultures/animals/independent experiments" etc. as applicable. All error bars need to be defined (e.g., s.d. or s.e.m.) together with a measure of center (e.g., mean or median) and precise n number, as noted above.

The type of repeat, precise "n", error bars, and measurable center are all defined in the figure legends.

18. Wherever statistical significance has been derived, precise P values should be provided if possible and appropriate. The type of statistical test used needs to be defined in the legend, whether they were one-sided or two-sided or whether adjustments were made for multiple comparisons.

Due to the nature of the data and the role that statistics play in their interpretability (which is minimal), we have opted not to report precise p-values in the text/legends. Precise p-values have been included in the source data files.

19. When representative experiments are shown (e.g. Fig 6 b,c), you should state in the legends how many times each experiment was repeated independently with similar results. Please indicate number of times experiments were repeated, number of images collected, etc. If space in the legends is limiting, this information can be included in the "Statistics and Reproducibility" subsection in Methods.

We have updated figure legends to reflect the "n" for representative experiments where this applies.

20. If applicable, the Methods should include a statistics section, listing statistical tests used, whether the test was one- or two-tailed, exact values

for both significant and non-significant *P* values where relevant; *F* values and degrees of freedom for all ANOVAs; and t-values and degrees of freedom for *t*-tests.

The only statistical test used in this study is a two-tailed Student's t-test and is indicated in each figure legend.

21. **Competing interests statement:** Please include a competing interests statement as a separate section after the Author Contributions, under the heading
"Competing interests", and enumerate any such circumstances there, or read: The authors declare no competing interests.

We have included this statement.

22. **Reporting Summary statement:** This should be placed after Online Methods section and read: Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

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23. **Data Availability statement:** This should be placed after Code Availability / Reporting Summary statement (before Methods-only references). We suggest that you list in this order:

- data deposited in public repositories, with accession codes or DOIs.
- data available as Source Data (e.g. "Source data are available with the paper online.")
- if any data can only be shared upon request, please specify what those data are and explain why.

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We have included this statement in the order suggested.

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The first author (Samuel Witus) and corresponding author (Dr. Rachel Klevit) have linked their ORCIDs to this manuscript.

Final Decision Letter:

23rd Dec 2020

Dear Dr. Klevit,

We are now happy to accept your revised paper "BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

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With kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology