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53BP1 Ablation Rescues Genomic Instability In Mice Expressing 'RING-less' BRCA1

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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 25 April 2016

As you will see, all referees acknowledge that the findings are interesting and novel. However, they also suggest a few more experiments and changes to the manuscript to further strengthen the study. Given the relatively small number of suggested changes and experiments and their relevance, I think that all of them should be addressed.

We would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point 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 this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 25,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In

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both cases, the entire materials and methods must be included in the main manuscript file. Please note that supplemental figures and tables are called expanded view (EV) now. These figures are integrated into the main text online and expand when clicked.

Please change the reference style to the numbered EMBO reports style that can be found in EndNote.

Regarding data quantification, can you please specify 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 in the respective figure legends? This information is currently incomplete and must be provided in the figure legends. Please also include scale bars in all microscopy images.

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.

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.

REFEREE REPORTS

Referee #1:

In this manuscript, Li et al. provide evidence that deletion of exon 2 of mouse Brca1 leads to the production of a 'RING-less' BRCA1 protein. Interestingly, despite missing its RING domain, and hence BRCA1's ability to interact and be stabilized by BARD1, this 'RING-less' protein is expressed at levels comparable to that of wild type BRCA1. RING-less BRCA1 localizes to DSB sites and is able to recruit RAD51 as well. The BARD1 protein is selectively lost in the exon2-deleted BRCA1 mutant cells. The authors show that Brca1ex2/ex2 primary mouse B cells are hypersensitive to olaparib and cisplatin and display genome instability despite RAD51 localization. Yet, the hypersensitivity to olaparib is rescued upon a co-deletion of Trp53bp1. Finally, the authors demonstrated that the 'RING-less' BRCA1 does not show an increased susceptibility to tumour development in the absence of 53BP1. Together, these findings suggest a role for the BRCA1 RING finger in promoting genome stability that is independent of its ability to promote RAD51 localization to DSB sites.

Overall, this is a very interesting set of observations that further suggests that the maintenance of genome stability by the BRCA1-BARD1 is complex and involves both HR-dependent and independent activities. The possibility that BARD1 may have a BRCA1-independent function during the mitotic cell cycle is also exciting and may open up new lines of investigation.

While I would like to see this manuscript published, there are a few substantive issues that need to be addressed first. I have the impression the manuscript was rushed a little, perhaps due to competing pressures.

Major Points

1. On page 10, the authors suggest that the presence of BARD1 in the floxed exon 2 cells reflects incomplete deletion of Brca1 exon2. However, in the same samples, full-length BRCA1 is undetectable, arguing against this explanation.

2. The authors demonstrate that in Brca1ex2/ex2Trp53bp1-/- MEFs, 'RING-less' BRCA1 is able to localize to irradiation-induced foci as demonstrated by the immunofluorescence of an individual cell. However, the author should present the quantitation of their immunofluorescence experiments.

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3. In the same vein, it would be important to assess whether RAD51 foci form in response to replication-associated DSBs (e.g. in response to CPT or olaparib treatment).

4. The authors report that 'RING-less' BRCA1 is able to load RAD51 at DNA break sites, yet there is still a deficiency in repair as Brca1Δ2/ Δ2 cells display signs of genomic instability. The authors suggest that the activity of the N terminus (RING domain) of BRCA1 is required for normal DNA repair, yet they do not perform any additional assays to suggest there are any defects in DNA repair. Does RAD51 loading by the 'RING-less' BRCA1 result in proper repair by homologous recombination? The authors could easily examine this using HR reporter assays.

5. Is the RING-less BRCA1 protein expressed in the tumors of the exon2-deleted animals? Is RAD51 still loaded at DSB sites in the tumors?

6. The authors really ought to test whether fork stability is impaired in the RING-less BRCA1-expressing cells.

Minor Points

1. The abstract is a little confusing since it does not refer to the previous work done on the Brca1ex2/ex2 mouse. It may be useful to clearly state the previous findings that Brca1ex2/ex2 mouse is embryonic lethal (Ludwig et al., 1997) in the abstract.

2. SinceLudwig et al., 1997 did not detect a RING-less BRCA1 in their studies, it may have been useful to show the BRCA1 levels in 53BP1+/+ Brca1ex2/ex2 cells in Figure 2.

3. On page 18 "(REFs)". The authors should include references.

Referee #2:

This manuscript is very interesting and merits publication in EMBO Reports. I have only one minor comment. In the first paragraph of the results section, the authors state:
"Although the Brca1-ex2 allele has previously been described as a null allele of Brca1 ...",
but no reference is provided. It is not clear to me, who claimed that the Brca1-ex2 allele is a null allele. The authors examined expression of the Brca1-ex2 allele in 53BP1-null background. Is the Brca1-ex2 allele expressed in a 53BP1-wt background? If Brca1-ex2/53BP1-wt cells cannot be obtained, then was it simply assumed that the Brca1-ex2 allele was a null allele? Given that this manuscript focuses on the Brca1-53BP1 functional interaction, these points should be made clear for the readers.

Referee #3:

In this study, Li and colleagues explore the effects of loss of the RING domain on BRCA1 function. They find that deletion of exon 2 results in production of a BRCA1 protein lacking the N-terminal RING domain. Loss of the RING domain causes destabilization of BARD1, genomic instability, a G2M checkpoint defect, and arrested spermatogenesis. Deletion of Trp53bp1 rescues genomic instability, and RING-less BRCA1 mutant mice with Trp53bp1 deletion form tumors at similar rates and have similar lifespan to exon 2 deletion heterozygotes, even in the context of Trp53 heterozygosity.

The findings presented here contribute to the understanding of BRCA1 function and characterize a BRCA1 mutation with genomic instability and normal Rad51 foci formation, indicating a potential role for BRCA1 and/or BARD1 in DNA repair after end resection and RAD51 loading. Although end resection and RAD51 loading appear to take place normally, genomic instability is still rescued by loss of 53BP1, suggesting that 53BP1 may have an effect on activities after RAD51 loading as well.

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Major Points:
The findings presented in this work are nuanced. This manuscript would benefit from careful rewriting to ensure that complexities of the data are well noted and that the main thrust of the manuscript is not obscured. For example, on p. 5, discussion of the Cao 2009, Bouwman 2010, and Bunting 2010 papers omits the facts that delta 11 shows no RAD51 foci (Cao), Bouwman showed the BRCA1 exon 5-13 knockout had no RAD51 foci, but they were rescued by 53bp1 knockout, and similarly, the 2010 Bunting paper showed RAD51 foci in the context of BRCA1 delta 11 were rescued by 53bp1 knockout. Thus, one function of the RING-less BRCA1 allele which is apparently absent in delta 11 or the exon 5-13 knockout is to allow RAD51 foci to form even in the absence of 53bp1 loss (figure 2C of the current paper). The discussion of BRCT domains has little relevance to the work presented in this manuscript and can be omitted from the discussion section so the main points are not obscured.

Another major point somewhat lost in the exposition is the finding that RING-less BRCA1 allows for RAD51 foci formation following IR but still promotes genomic instability that can be rescued by ablation of 53BP1, indicating that BRCA1 and/or BARD1 might have a function after end resection and RAD51 loading in DSB repair. Further, the finding that 53bp1 rescues genomic instability seen with the RING-less allele points to a function of 53bp1 downstream of RAD51 loading. These would appear to be central points in this manuscript, and should be made much more clearly. Additional data would strengthen these points: RING-less BRCA1 function might be further studied by using a DSB repair reporter (Maria Jason assay) in RING-less BRCA1 cells to assay DSB repair more directly, or at least studied by gamma-H2AX persistence and comet assays with and without 53bp1 loss to try to elucidate more details of these new functions of BRCA1 and 53BP1. It would also be of interest to have the authors amplify their comments relative to the relationship of the RING-less allele to known BRCA1 clinical mutations. Do the authors think that RING domain mutations might be different in clinically significant ways from other BRCA1 mutations? For example, is it possible based on their data that the platinum agents might be a better choice in patients with tumors harboring the common C61G mutation that PARP Inhibitors, since 53bp1 loss would not confer resistance?

Points about BARD1 should be augmented with additional data that show causation rather than correlation, or the conclusions drawn about the function of BARD1 should be less strongly worded. (For example, in the abstract the authors state: "These results indicate that BARD1 has an important role in DNA double-strand break repair that is independent of RAD51 loading." The data presented do not show this conclusively as the effects of low BARD1 levels and RING-less BRCA1 cannot be separated; either experiments should be presented that show that a stabilized or overexpressed BARD1 rescues these defects, or conclusions about BARD1 should be stated more cautiously, as in, they MAY indicate...)

In the manuscript text, figure legends, and in the figures themselves, Trp53bp1 status should be clearly stated. As it currently stands, it is much too hard for the reader to figure out this status.

Minor Points:
Text Corrections: Zhu et al 2011 is referenced on page 5 but does not appear in the references list. The references for BRCA1 and stalled for replication protection on page 18 should be added.

1st Revision - authors' response 22 July 2016

Thank you for the opportunity to resubmit our manuscript (EMBOR-2016-40497-T, “53BP1 Ablation Rescues Genomic Instability In Mice Expressing ‘RING-less’ BRCA1”). I was pleased that the reviewers appreciated the significance of our work. I am excited about our discovery of ‘RING-less’ BRCA1, which offers an opportunity to better understand how this important tumor suppressor works. I was glad that the reviewers agreed that this finding is “very interesting” and “exciting”. I agree with most of the reviewers’ comments, including their criticisms of the deficiencies of the previous submission. I am now pleased to offer a revised version. Based on the reviewers’ comments, we have carried out a substantial number of new experiments, which I believe considerably strengthen the report. I have also rewritten the text to more clearly articulate the primary significance of the work.

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I decided to reformat the manuscript in the shorter 'scientific report' style, which I think helps to achieve a clearer, more succinct report, and which best fits the amount of data. I have endeavored to conform to all the journal's requirements for formatting and data presentation.

As you are aware, while we were preparing this manuscript, we became aware of two other groups who had results pointing to a role for N-terminal truncated forms of BRCA1 in the growth of cancer cells. Although there are differences in the experimental systems and goals of our studies, our findings were sufficiently well aligned that we decided to make our first submission of this manuscript together with reports from the other groups. The other reports got more favorable reviews from the original submission, and my understanding is that they are now close to publication. I hope you will take this into consideration and try to come to a quick decision so that we can try to get our work out as close as possible to these other closely-related papers.

I have included a point-by-point response to the reviewers' comments. I hope that they will agree that the revised manuscript is worthy of publication in 'EMBO Reports'. Thanks again for your consideration.

REVIEWER #1

Major Points

1. On page 10, the authors suggest that the presence of BARD1 in the floxed exon 2 cells reflects incomplete deletion of Brca1 exon2. However, in the same samples, full-length BRCA1 is undetectable, arguing against this explanation.

The reviewer is correct that the data do not support the proposed explanation, so we have removed this statement. The level of BARD1 in the floxed exon 2 cells is moreover not substantially greater than that seen in ex2/ex2;53BP1-/- cells (cf Fig 1B), so our previous statement served only to confuse the reader.

2. The authors demonstrate that in Brca1ex2/ex2Trp53bp1-/- MEFs, 'RING-less' BRCA1 is able to localize to irradiation-induced foci as demonstrated by the immunofluorescence of an individual cell. However, the author should present the quantitation of their immunofluorescence experiments.

We have quantified the immunofluorescence experiments in two different ways, focusing on the proportion of cells showing both RAD51 and BRCA1 foci, and the extent of colocalization of RAD51 and BRCA1. This data is present as new panels, Fig 1D-E.

3. In the same vein, it would be important to assess whether RAD51 foci form in response to replication-associated DSBs (e.g. in response to CPT or olaparib treatment).

We have done this experiment with both CPT and olaparib, and find that RAD51 foci are robustly induced in response to both drugs in both the WT and mutant cells. This data is presented as a new panel, Fig 2D.

4. The authors report that 'RING-less' BRCA1 is able to load RAD51 at DNA break sites, yet there is still a deficiency in repair as Brca1Δ2/ Δ2 cells display signs of genomic instability. The authors suggest that the activity of the N terminus (RING domain) of BRCA1 is required for normal DNA repair, yet they do not perform any additional assays to suggest there are any defects in DNA repair. Does RAD51 loading by the 'RING-less' BRCA1 result in proper repair by homologous recombination? The authors could easily examine this using HR reporter assays.

This is a very good point, and gets to the question of why cells expressing RING-less BRCA1 show genomic instability? In the original manuscript, we did not have any positive data indicating a specific defect in repair. Our subsequent experiments, presented in the revised version, showed that cells expressing RING-deleted versions of BRCA1 show reduced ability to restart replication forks after replication stress induced by hydroxyurea treatment. This allows us to make a positive statement about a potential role for the BRCA1 RING domain in maintaining genomic integrity. This data is presented as a new Figure, Fig 3. By measuring the recovery of gamma-H2AX after

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ionizing radiation, we did not find any evidence of a substantial defect in double-strand break repair in the cells expressing RING-less BRCA1. This data is presented as the new panel Fig 2E. As such, we propose that genomic instability arises because of failures in replication as opposed to DNA repair per se.

We did aim to directly test homologous recombination using HR reporter assays. The experiment is not totally straightforward, because we need to both knock down endogenous BRCA1 in suitable reporter cells and then express exogenous WT or mutant BRCA1 at a suitable level. It also requires a switch from mouse to human cells. We were able to get the reporter assays working, knock down endogenous BRCA1 using custom siRNA oligos, and generate constructs for RING-deleted BRCA1. Unfortunately, despite running many optimization experiments, we could not get the BRCA1 construct to express at a high enough level to give useful data. As an alternative, we used the sister chromatid assay to measure HR efficiency in cells expressing RING-less BRCA1. This assay showed no significant defect in the frequency of recombination events in the mutant cells. We hope that the reviewer will find this alternative experimental approach acceptable.

5. Is the RING-less BRCA1 protein expressed in the tumors of the exon2-deleted animals? Is RAD51 still loaded at DSB sites in the tumors?

After receiving the reviews, we ran a Western blot to test BRCA1 protein expression in one animal that developed a tumor, and found that RING-less BRCA1 was present in the sample. Unfortunately all our other animals from the longitudinal study were euthanized a long time ago and we don't have protein lysates to do more Westerns. We did fix tumor samples from the test animals post mortem, so we tried to quantify BRCA1 levels by immunohistochemistry, but our anti-mouse BRCA1 antibody did not work well enough for this assay. As we only have data from one mouse and it would take months/years to age more mice to the point where they get tumors, we prefer not to make any statements about BRCA1 protein levels in the tumors.

6. The authors really ought to test whether fork stability is impaired in the RING-less BRCA1-expressing cells.

Based on the reviewer's excellent suggestion, we adapted the DNA combing assay following the description of Schwab and Niedzwiedz (JoVE, 2011) to test replication fork stability. We found an increase in the frequency of stalled forks after hydroxyurea treatment in cells expressing RING-less BRCA1. This result provides new insight into the function / activity of the RING domain in maintenance of genomic integrity. We were previously focused on the idea that there must be some defect in double-strand break repair, but our assays did not support this hypothesis (see also point #4, above). It seems that the RING domain is more important for replication. Having worked up this protocol, we now hope to do further experiments to investigate the role of RING mutants in ensuring efficient replication.

Minor Points

1. The abstract is a little confusing since it does not refer to the previous work done on the Brca1ex2/ex2 mouse. It may be useful to clearly state the previous findings that Brca1ex2/ex2 mouse is embryonic lethal (Ludwig et al., 1997) in the abstract.

We have now included a clear statement in the abstract that the Brca1ex2/ex2 mouse is embryonic lethal.

2. SinceLudwig et al., 1997 did not detect a RING-less BRCA1 in their studies, it may have been useful to show the BRCA1 levels in 53BP1+/+ Brca1ex2/ex2 cells in Figure 2.

We have now included this data as Figure EV1A, linked to Figure 2. In general, we find that 53BP1 status does not affect the stability of RING-less BRCA1. Our data may vary from earlier reports because we are using a different antibody, or because BRCA1 levels are higher in actively dividing B cells from Brca1ex2/ex2;53bp1-/- mice.

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3. On page 18 "(REFs)". The authors should include references.

This reference has now been included.

REVIEWER #2

1. In the first paragraph of the results section, the authors state:
"Although the Brca1-ex2 allele has previously been described as a null allele of Brca1 ...",
but no reference is provided. It is not clear to me, who claimed that the Brca1-ex2 allele is a null allele. The authors examined expression of the Brca1-ex2 allele in 53BP1-null background. Is the Brca1-ex2 allele expressed in a 53BP1-wt background? If Brca1-ex2/53BP1-wt cells cannot be obtained, then was it simply assumed that the Brca1-ex2 allele was a null allele?

We have now included a reference to a review about BRCA1 mouse models from Jos Jonkers (Evers & Jonkers, 2006) that explicitly refers to BRCA1-ex2 as a null. Brca1-ex2 was considered a null partly because of the very early embryonic lethality phenotype of the homozygous mice and also because no protein was expected to be produced based on the structure of the targeted allele. We have tried to clarify this further in the revised text. The assumption that no protein is expressed appeared to be validated by a report by McCarthy et al (MCB 2003), which failed to detect BRCA1 protein in E9.5 embryos from Brca1ex2/ex2;p53-/- mice. However as these embryos are non-viable, we would argue that they may not express high levels of BRCA1 protein.

REVIEWER #3

Major Points:

1. The findings presented in this work are nuanced. This manuscript would benefit from careful rewriting to ensure that complexities of the data are well noted and that the main thrust of the manuscript is not obscured.

We have substantially rewritten the manuscript, which, together with the new data, has hopefully made the report much clearer. As the reviewer advised, we have now clearly stated that the delta 11 and exon5-13 knockout models have a defect in IR-induced RAD51 foci formation (page 8), and we contrast this phenotype with the apparently-normal RAD51 foci formation in our delta 2 cells. We have also removed essentially all references to the BRCA1 BRCT domain, which, as the reviewer noted, were not relevant to the current study.

2. Another major point somewhat lost in the exposition is the finding that RING-less BRCA1 allows for RAD51 foci formation following IR but still promotes genomic instability that can be rescued by ablation of 53BP1, indicating that BRCA1 and/or BARD1 might have a function after end resection and RAD51 loading in DSB repair. Further, the finding that 53bp1 rescues genomic instability seen with the RING-less allele points to a function of 53bp1 downstream of RAD51 loading.

Based on our most recent data, obtained with the DNA combing assay (Figure 3 in the revised manuscript), we propose that the BRCA1 RING domain and 53BP1 are in fact involved in maintenance of stability of replication forks. This is not necessarily incompatible with a late role in HR, however, we have further observed that gamma-H2AX foci recover normally and sister chromatid exchanges form at the regular rate in cells expressing RING-less BRCA. (This data forms new panels Fig 2 E and F.) Although we do not rule out a role for the BRCA1 RING domain in double-strand break repair, our data are more consistent with a role in maintaining genomic integrity by ensuring efficient replication. These findings are explained in the latest version of the manuscript.

3. Additional data would strengthen these points: RING-less BRCA1 function might be further studied by using a DSB repair reporter (Maria Jason assay) in RING-less BRCA1 cells to assay DSB repair more directly, or at least studied by gamma-H2AX persistence and comet assays with and without 53bp1 loss to try to elucidate more details of these new functions of BRCA1 and 53BP1

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I agree that a Maria Jasin-type HR reporter assay would potentially be informative for this study (*see also response to Reviewer #1, point 4*). We have spent a lot of time trying to get the experiment to work. To model expression of RING-less BRCA1 in the human reporter cells, we need to knock down endogenous BRCA1, introduce exogenous WT/ mutant BRCA1 and then run the assay. We were able to get the reporter assays working, knock down endogenous BRCA1, and generate constructs for RING-deleted BRCA1. Unfortunately, at this time our transfections have not resulted in expression of the BRCA1 constructs at a sufficiently high enough level to give useful data. As an alternative assay for HR, we quantified sister chromatid exchanges in cells expressing WT or RING-less BRCA1. This assay showed no significant defect in the frequency of recombination events in the mutant cells. We also looked at gamma-H2AX persistence, which again did not show a difference between the WT and mutant cells. As mentioned for point #2, above, our additional data, presented in the new figure (Fig 3), support a role for the BRCA1 RING domain in replication fork stability instead of in repair of DNA double-strand breaks. We have updated the narrative to reflect these findings.

4. It would also be of interest to have the authors amplify their comments relative to the relationship of the RING-less allele to known BRCA1 clinical mutations. Do the authors think that RING domain mutations might be different in clinically significant ways from other BRCA1 mutations? For example, is it possible based on their data that the platinum agents might be a better choice in patients with tumors harboring the common C61G mutation that PARP Inhibitors, since 53bp1 loss would not confer resistance?

Based on our results, I would expect RING domain mutations to have a different effect from other *BRCA1* mutations. Different parts of the BRCA1 protein control seem to control different cellular processes, so in cases where a mutant protein isoform is expressed, that isoform can mediate a subset of activities relevant for cell survival. Tumor cells expressing a 'RING-less' BRCA1 protein would still be able to load RAD51 at DNA break sites, even if the mutant protein did not fully support restart of replication after replication stress. RING-mutated BRCA1 isoforms could therefore act as hypomorphic forms of BRCA1 and potentially contribute to resistance to chemotherapy.

These points are discussed in a some more detail in the new version of the manuscript. I am also aware of two other groups who have specific data indicating that N-terminal-truncated forms of BRCA1 can mediate chemoresistance. These manuscripts are currently under revision / in press, and I hope to discuss with the editor about how I might be able to cite their work if our manuscript is selected for publication.

5. Points about BARD1 should be augmented with additional data that show causation rather than correlation, or the conclusions drawn about the function of BARD1 should be less strongly worded.

It totally agree with this point. Right now, we are using several experimental strategies to test whether BARD1 can be stabilized at DNA break sites in cells expressing RING-less BRCA1. Hopefully this will allow us to distinguish whether it is loss of the RING domain *per se* or loss of BARD1 that causes genomic instability. As these experiments are not complete, we have carefully rechecked our claims about BARD1 to ensure that any statements made are fully supported by the existing data.

6. In the manuscript text, figure legends, and in the figures themselves, Trp53bp1 status should be clearly stated. As it currently stands, it is much too hard for the reader to figure out this status.

We have annotated *Tr53bp1* status to all the figures and apologize for the confusion.

Minor Points:

1. Text Corrections: Zhu et al 2011 is referenced on page 5 but does not appear in the references list. The references for BRCA1 and stalled for replication protection on page 18 should be added.

These references have been added correctly in the latest version.

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2nd Editorial Decision 22 August 2016

Thank you for the submission of your revised manuscript to our journal. We have now received the enclosed report from referee 1 who was asked to assess it. I am happy to tell you that s/he supports the publication of your study now and we can therefore in principle accept it.

Regarding statistics, Figs 1D,E; 2D-F; 3B-D; 5D state n=2, in which case no statistics can be calculated. Please either repeat the experiments at least one more time so n=3 or remove the error bars and p-values and instead show all single data points along with their mean in the graphs. It would be much better to repeat the experiments one more time though.
Fig EV1B does not specify "n" nor the error bars, please add this information.
The text in the legend for Fig 5C seems to be truncated, please check.

I look forward to seeing a final version of your manuscript as soon as possible.

REFEREE REPORTS

Referee #1:

I am satisfied with the authors' revised manuscript. The manuscript deserves to be published especially in light of the two JCI papers on the contribution of RING-less isoforms of BRCA1 to therapy resistance.

2nd Revision - authors' response 08 September 2016

Thank you for conditionally agreeing to accept our manuscript (EMBOR-2016-40497-T, “53BP1 Ablation Rescues Genomic Instability In Mice Expressing ‘RING-less’ BRCA1”) for publication in *EMBO Reports*.

I am now submitting new versions of the text and figures taking account of the recommendations in your message of August 22. I have reformatted a number of panels to conform with guidelines on presentation of statistics. I have made some corrections to the text, including adding discussion of two recent, highly-relevant publications in the *Journal of Clinical Investigation* that discussed ‘RING-less’ BRCA1.

I have also included summary text including bullet points highlighting key results, and a synopsis image.

I hope you find the materials suitable. I will be happy to make any other modifications that you think are appropriate. Thanks again for considering our work.

3rd Editorial Decision 09 September 2016

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

Corresponding Author Name: Samuel F Bunting
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2016-42497-T

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.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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?	In each experiment, a sample size was chosen to determine statistical significance at a level of P<0.05. Owing to the cost of breeding animals of the requisite genotypes, we had to compromise on statistical power in some experiments, and it is possible we may have missed small differences in effect size.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal survival studies, a minimum of 20 animals per cohort was considered sufficient to provide sufficient confidence about effect size.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Only healthy animals were used for cellular experiments. For the aging study, animals were excluded if they were injured after fighting or for unexpected death in the case of, for example, cage flooding. These criteria were set up in advance.
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.	No randomization was used.
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 analysis of the RAD51 immunofluorescence experiment, images files were anonymized and randomized by the PI, and then scored by a different staff member. This process of blind scoring of the images was intended to prevent subjective biases in analysis.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done. Animals were kept in cages with their genotypes marked.
5. For every figure, are statistical tests justified as appropriate?	We have included statistical tests as appropriate for each panel.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	To the greatest extent possible, we aimed to check that data followed a normal distribution, but in several cases this was not possible because of low N.
Is there an estimate of variation within each group of data?	An estimate of variation, usually standard deviation, is annotated for each data set where possible.
Is the variance similar between the groups that are being statistically compared?	Variance was typically similar between groups. In some experiments there was a difference in variance between groups; this is clearly indicated.

C- Reagents

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://jij.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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).	Catalog numbers for all antibodies used are listed in the 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.	MEF cell lines were generated by the authors. They have not been authenticated, but we have no reason to suspect mycoplasma contamination.

* 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.	Descriptions of mice used are listed as applicable in the text.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This statement is included in the methods section.
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.	Confirmed.

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 accession codes for deposited data. See author guidelines, under '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. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	We have not included primary data at this time.
22. 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

23. 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.	N/A