JCB

BIK ubiquitination by the E3 ligase Cul5-ASB11 determines cell fate during cellular stress

Fei-Yun Chen, Min-Yu Huang, Yu-Min Lin, Chi-Huan Ho, Shu-Yu Lin, Hsin-Yi Chen, Mien-Chie Hung, and Ruey-Hwa Chen

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Review Timeline:

Submission Date:	2019-01-30
Editorial Decision:	2019-03-04
Revision Received:	2019-06-04
Editorial Decision:	2019-06-20
Revision Received:	2019-06-22

Monitoring Editor: Nika Danial

Scientific Editor: Marie Anne O'Donnell

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.)

DOI: https://doi.org/10.1083/jcb.201901156

March 4, 2019

Re: JCB manuscript #201901156

Dr. Ruey-Hwa Chen
Academia Sinica
128 Academia Rd., Sec II, Nankang
Taipei 11529

Dear Dr. Chen,

Thank you for submitting your manuscript entitled "BIK ubiquitination controls life-death fate of cellular stress responses and anti-tumor activity". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

All three reviewers emphasize the main finding that ER stress and DNA damage responses can differentially regulate BIK levels via ASB11 is interesting as little is known about the regulation of BIK. The reviewers have recommended some further experimental work, which we agree is necessary to support the main claims. If Reviewer #2's request to provide more substantive evidence that BIK is modified with K48-linked ubiquitin chains in a ASB11 activity dependent manner for degradation via the proteasome can be addressed, we consider it less necessary to provide mass spec data that the proposed acceptor lysines are modified, provided the mutants maintain interaction with ASB11.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations

are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

The typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Nika Danial, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

This is a very interesting and well supported manuscript by Chen et al that uncovers new details about the regulation of the BH3-only protein BIK. While BIK has been studied for over 15 years, it is still one of the most poorly understood BH3-only proteins. As such, the questions that these authors address are of great interest to the cell death community.

First, the authors discover that the E3 ligase CRL5/ASB11 plays a key role in the ubiquitination and degradation of BIK.

Second, they show that under ER stress ASB11 is activated by the IRE1/XBP1 arm of the UPR and is a direct transcriptional target of XBP1. The provide evidence that XBP1-ASB11-mediated degradation of BIK is important for delaying cell death during the early phases of ER stress.

Third, they show that genotoxic agents downregulate ASB11 through a p53 dependent mechanism to stabilize BIK as part of the apoptotic response to DNA damage.

Finally, they show that preventing BIK turnover through IRE1 inhibition can enhance tumor cell killing in xenograft models of triple negative breast cancer.

Overall, the experiments are well controlled and the results convincing.

They have done a great deal of work here.

However, there are a few important things missing that should be done to further support their conclusions.

1) In Figure 3A, they should show ASB11 protein levels (not just mRNA levels) under each of these conditions.

2) In Figure 4A, they should test whether XBP1s overexpression is sufficient to increase ASB11 protein expression and reduce BIK protein levels.

3) In Figure 4F, they should explain why ASB11 knockdown does not seem to effect total BIK levels in the lysate.

4) In Fig 5E-F, they should show ASB11 protein levels (not just transcript levels).

5) The cell death experiments in Figure 6 should be supplemented by testing the effects of BIK knockdown (not just overexpression). In reality, overexpression of any apoptotic protein is likely to overcome the protective effects of ASB11 overexpression on DNA damage-induced cell apoptosis. However, this does not mean that it is regulating this event when expressed under physiological levels.

6) In Figure 7, the use of pharmacological inhibitors against IRE1 should be supplemented with IRE1 knockout and XBP1s overexpression conditions (to control for off target effects of the IRE1 inhibitors).

Reviewer #2 (Comments to the Authors (Required)):

In the manuscript entitled 'BIK ubiquitination controls life-death fate of cellular stress responses and anti-tumor activity' by Chen et al., the authors demonstrated that the E3 ligase Asb11, which is known to reside on ER, regulates turnover of BIK (Bcl2-interacting killer) by ubiquitination. BIK protein stability was known to be regulated by proteasome based on proteasomal inhibitor (Bortezomib) assays; however, the ubiquitin E3 ligase responsible for this event was not known until this study. The regulation of Asb11 is carried out by ER stress-induced XBP1s. The focus of the study is interesting, and the study provides a new aspect on the functions of BIK which depends on its protein degradation. The manuscript is well written, and the data provided are mostly convincing. There are some points as below, which need to be shown to improve the manuscript and to be published.
Major points:
1. Figure S4. BIK has only two Lys residues; thus, the authors assumingly targeted K115 and K160 as ubiquitination sites to mutate. However, it is very important to confirm these sites to be ubiquitinated in cells by mass spectrometry since mutation could lead to unexpected effects. For example, mutations at K115R K160R in BIK may affect the recognition by ASB11 thus, ubiquitination of BIK is no longer happening.
2. Figure 2. and Figure 4B. Even though the previous studies by Li et al 2008 and Zhu et al 2005 showed that the proteasome inhibitor Bortezomib stabilizes BIK in cells, it remains important to examine the effect by MG132 in the experimental conditions used in this study to obtain direct evidence of proteasomal degradation of BIK.
3. Ubiquitination assays in general: It is important to examine which linkage types of ubiquitin chains

are conjugated on BIK. At least the authors need to confirm that BIK is ubiquitinated by K48-linked ubiquitin chains by using as specific antibody against K48 chains.
4. Ubiquitination assays in general: Since all the ubiquitination assays were performed in cells, it is not clear if the ubiquitination event is dependent on the Asb11 catalytic activity, or on direct effect by Asb11. To this end, it is necessary to test BIK ubiquitination using an Asb11 catalytic inactive mutant. In this way, at least the evidence of BIK ubiquitination which depends on Asb11 catalytic activity (indirectly or directly) could be provided.
5. Fig S5A-D: To conclude BIK-DD undergoes ASB11- and ER stress-dependent degradation as stated in page 15, the BIK-DD Lys mutant (the two Lys sites tested in Figure S4) needs to be examined to show that the mutant is no longer ubiquitinated and is stable.

Reviewer #3 (Comments to the Authors (Required)):

This work aim to unravel the degradation mechanism of BIK, a BH3-only protein of the BCL2 family and its pro-apoptotic activity under ER/Genotoxic stress conditions. The authors were capable to dissect very convincingly the molecular mechanisms of ubiquitin-mediated degradation of BIK, the specific role of the ASB11 adaptor and its transcriptional regulation mediated by the UPR mediator XBP1. Furthermore, the authors show the implications of the expression of P53wt in the transcriptional repression of ASB11 and its consequences on cell viability and apoptosis under genotoxic stress. Finally, the authors go further and showed the antitumor potential of mutant-BIK expression combined with a pharmacological IRE1a inhibitor in 2 preclinical breast cancer models. I have only minor concerns and the article is suitable for publication in JCB. As a final comment, the role of P53 in vitro (cancer cell lines) and its mutational status are important to be in consideration (mainly in the discussion section), because an important proportion of solid tumors contain inactivating mutations of P53, that disrupt its canonical function. Also, several studies have described new roles of this mutant proteins that can be in oppositions with P53 WT.
Minor concerns
Figure 1. The molecular weight of BIK (22 to 25 KD) doesn't appear in the gel..why? For example in the IP experiments Fig 1E
Figure 1. Any know interactor for ASB11 as positive control?
Figura 2. What is the time of exposure with mg132 used in the experiments?
Figure 2d. BIK expression doesn't appear in the non-treated condition, why?
Figure 3. As an internal control, authors could pretreat the cell with IRE1 inhibitor and then incubate with an ER stressor as tunicamycin ...then check the BIK expression by WB
Add a positive control for XBP1 binding sites (promoter of canonical targets) for luciferase and ChIP experiments.
Figure 5. What is the expression of ASB11 in cell lines with P53 WT, P53 mutant (in your work MDA-MB157 and MDA-MB468) and P53 KO. Are the P53 KO cells similar to P53 mutant,? Please discuss.
Figure 5b. It's very interesting that in P53 KO cells the genotoxic stress increases the ASB11 expression in opposition to P53 wt cells, please discuss.
Figiure 5c. The effectson BIK expression in shRNA ASB11 cells under genotoxic condition it's very subtle and similar to control experiments, please discuss.
Figure 7a. What is the statistical significance of this figure?
Please discuss the effect of STF on the IRE1/XBP1 axis inhibition and the possible BIK stabilization and cellular apoptosis.
Finally in tumor cells, the inhibition of IRE1 activity and its consequences on BIK stability could be independent of P53 expression/status because the Hs578T cell lines using in the preclinical models have a point mutation in the P53 gene, please discuss.

Responses to reviewers' comments

Reviewer #1

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1) In Figure 3A, they should show ASB11 protein levels (not just mRNA levels) under each of these conditions.

Ans: We provide new data in Fig. 3B showing that ASB11 protein level is also induced by ER stressors.

2) In Figure 4A, they should test whether XBP1s overexpression is sufficient to increase ASB11 protein expression and reduce BIK protein levels.

Ans: As requested by reviewer, this piece of new data is shown in Fig. S2I. XBP1s overexpression indeed increases ASB11 and reduces BIK protein levels

3) In Figure 4F, they should explain why ASB11 knockdown does not seem to effect total BIK levels in the lysate.

Ans: In all in vivo ubiquitination assays performed in this study, cells were treated with proteasome inhibitor MG132 to prevent the degradation of ubiquitinated

proteins. This is described in the Materials and Methods section under the title of "Ubiquitination assays". As a consequence, substrate, i.e., BIK, degradation is prevented.

4) In Fig 5E-F, they should show ASB11 protein levels (not just transcript levels).
Ans: As requested by reviewer, the ASB11 protein level changes are now shown in Fig. 5E and 5F.

5) The cell death experiments in Figure 6 should be supplemented by testing the effects of BIK knockdown (not just overexpression). In reality, overexpression of any apoptotic protein is likely to overcome the protective effects of ASB11 overexpression on DNA damage-induced cell apoptosis. However, this does not mean that it is regulating this event when expressed under physiological levels.
Ans: We fully agree with the reviewer. In Fig. 6A, we now show that BIK knockdown attenuated doxorubicin-induced apoptosis, indicating a physiological role of BIK in this cell death paradigm.

6) In Figure 7, the use of pharmacological inhibitors against IRE1 should be supplemented with IRE1 knockout and XBP1s overexpression conditions (to control for off target effects of the IRE1 inhibitors).
Ans: These two pieces of new data are included in Fig. S5H and S5I. The ability of IRE1α inhibitor to enhance the tumor-killing effect of BIKDD is indeed abolished by IRE1α knockdown or XBP1s overexpression. Thus, these data indicate the specificity of IRE1α inhibitor.

Reviewer #2
In the manuscript entitled 'BIK ubiquitination controls life-death fate of cellular stress responses and anti-tumor activity' by Chen et al., the authors demonstrated that the E3 ligase Asb11, which is known to reside on ER, regulates turnover of BIK (Bcl2-interacting killer) by ubiquitination. BIK protein stability was known to be regulated by proteasome based on proteasomal inhibitor (Bortezomib) assays; however, the ubiquitin E3 ligase responsible for this event was not known until this study. The regulation of Asb11 is carried out by ER stress-induced XBP1s. The focus of the study is interesting, and the study provides a new aspect on the functions of BIK which depends on its protein degradation. The manuscript is well written, and the data provided are mostly convincing. There are some points as below, which need to be shown to improve the manuscript and to be published.

Major points:

1. Figure S4. BIK has only two Lys residues; thus, the authors assumingly targeted K115 and K160 as ubiquitination sites to mutate. However, it is very important to confirm these sites to be ubiquitinated in cells by mass spectrometry since mutation could lead to unexpected effects. For example, mutations at K115R K160R in BIK may affect the recognition by ASB11 thus, ubiquitination of BIK is no longer happening.

Ans: We provide Mass Spectrometry data for the ubiquitination in K115 of BIK in ASB11 overexpressing cells (Fig. S4A). In addition, we showed that the K115R/K160R (2KR) mutant of BIK can still bind ASB11 (Fig. S4C). Thus, the inability of this 2KR mutant to be ubiquitinated by ASB11 is not due to a defect in binding ASB11.

2. Figure 2. and Figure 4B. Even though the previous studies by Li et al 2008 and Zhu et al 2005 showed that the proteasome inhibitor Bortezomib stabilizes BIK in cells, it remains important to examine the effect by MG132 in the experimental conditions used in this study to obtain direct evidence of proteasomal degradation of BIK.

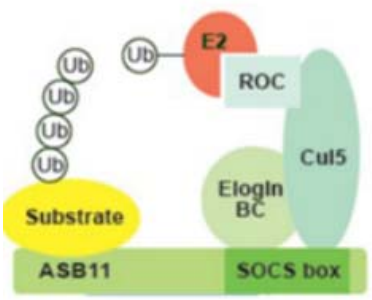
Ans: We show in Fig. 2B that MG132 treatment abolishes ASB11-induced BIK downregulation. Furthermore, in Fig. 4B, we add new data demonstrating that MG132 blocks ER stress-induced BIK downregulation.

3. Ubiquitination assays in general: It is important to examine which linkage types of ubiquitin chains are conjugated on BIK. At least the authors need to confirm that BIK is ubiquitinated by K48-linked ubiquitin chains by using as specific antibody against K48 chains.

Ans: We fully agree with the reviewer. In Fig. 1G, we provide new data showing that ASB11 increases BIK K48 ubiuqitination.

4. Ubiquitination assays in general: Since all the ubiquitination assays were performed in cells, it is not clear if the ubiquitination event is dependent on the Asb11 catalytic activity, or on direct effect by Asb11. To this end, it is necessary to test BIK ubiquitination using an Asb11 catalytic inactive mutant. In this way, at least the evidence of BIK ubiquitination which depends on Asb11 catalytic activity (indirectly or directly) could be provided.

Ans: ASB11 is a substrate adaptor of Cul5 ubiquitin ligase. Each Cul5-family E3 ligase complex contains five subunits, the catalytic subunit ROC2, the scaffold protein Cul5, the linker proteins ElonginB and ElonginC, and one of many substrate adaptors (see Figure on the right; for review see Okumura et al., Cell Div 11:1, 2016). Therefore, ROC2, rather than ASB11, possesses the catalytic activity. However, ROC2 is shared by Cul2 and Cul5 ubiquitin ligase families, which comprise >100 different members. Thus, perturbing the catalytic activity of ROC2 cannot provide specific evidence for a catalytic activity-dependent role of ASB11-based Cul5 ubiquitin ligase. Since the SOCS box present in most Cul5 substrate adaptors, including ASB11, is responsible for the binding of ElonginB/C and Cul5 and the subsequent recruitment of ROC2, we used ASB11△SOCS mutant to address a specific and catalytic activity-dependent role of ASB11-based Cul5 complex. This strategy is widely used in the Cullin ubiquitin ligase filed. Importantly, data shown in Fig. 1E and Fig. 2A indicate that ASB11△SOCS mutant cannot promote BIK ubiquitination and degradation.



5. Fig S5A-D: To conclude BIK-DD undergoes ASB11- and ER stress-dependent degradation as stated in page 15, the BIK-DD Lys mutant (the two Lys sites tested in Figure S4) needs to be examined to show that the mutant is no longer ubiquitinated and is stable.

Ans: We provide new data in Fig. S5E, F indicating that BIKDD(2KR) cannot be ubiquitinated and degraded by ASB11.

Reviewer #3

This work aim to unravel the degradation mechanism of BIK, a BH3-only protein of the BCL2 family and its pro-apoptotic activity under ER/Genotoxic stress conditions. The authors were capable to dissect very convincingly the molecular mechanisms of ubiquitin-mediated degradation of BIK, the specific role of the ASB11 adaptor and its transcriptional regulation mediated by the UPR mediator XBP1. Furthermore, the authors show the implications of the expression of P53wt in the transcriptional repression of ASB11 and its consequences on cell viability and apoptosis under genotoxic stress. Finally, the authors go further and showed the antitumor potential of mutant-BIK expression combined with a pharmacological IRE1a inhibitor in 2 preclinical breast cancer models. I have only minor concerns and the article is

suitable for publication in JCB. As a final comment, the role of P53 in vitro (cancer cell lines) and its mutational status are important to be in consideration (mainly in the discussion section), because an important proportion of solid tumors contain inactivating mutations of P53, that disrupt its canonical function. Also, several studies have described new roles of this mutant proteins that can be in oppositions with P53 WT.

Ans: As described below, we included discussions on the role of wild type and mutant p53 in regulating ASB11 expression and BIK (or BIKDD) expression in several places of the Discussion section. Improtantly, we did provide evidence that certain p53 mutant fails to regulate ASB11 and functions as a loss-of-function mutant in this aspect.

Minor concerns

Figure 1. The molecular weight of BIK (22 to 25 KD) doesn't appear in the gel..why? For example in the IP experiments Fig 1E

Ans: Figure 1E is the in vivo ubiquitination assay. Since the assay is to pull down total cellular ubiquitinated proteins followed by BIK Western blot, only ubiquitinated BIK can be detected by this method. The molecular weight of ubiquitin is 8 KD. Therefore, the lowest band on gel (appearing in between 25 to 35 KD) represents monoubiquitinated BIK.

Figure 1. Any know interactor for ASB11 as positive control?

Ans: ASB11 is a poorly studied protein. The only other known substrate of ASB11-Cul5 complex is the ER-resident protein ribophorin1. However, we do not have reagents to reconfirm this interaction. Since this is unrelated to our study and since we have provided a substantial amount of data indicating a direct and specific role of ASB11 in BIK regulation, we consider this positive control as unnecessary.

Figure 2. What is the time of exposure with mg132 used in the experiments?

Ans: We add the treatment time (16 h) in the legend of Fig. 2B.

Figure 2d. BIK expression doesn't appear in the non-treated condition, why?

Ans: The blot was exposed for a short time and therefore the low level of endogenous BIK is not detectable in this blot. This piece of data indicates that BIK is a highly labile protein, consistent with previous reports.

Figure 3. As an internal control, authors could pretreat the cell with IRE1 inhibitor and then incubate with an ER stressor as tunicamycin ...then check the BIK expression

by WB

Ans: We add this control data in Fig. S2D, IRE1 inhibitor pretreatment indeed blocks tunicamycin-induced BIK downregulation.

Add a positive control for XBP1 binding sites (promoter of canonical targets) for luciferase and ChIP experiments.

Ans: These control data are included in Fig. 3F and Fig. 3G. We used ERSEI reporter, a widely used reporter for determining XBP1s transcriptional activity, for luciferase assay, and *EDEM1* gene, a well-known target of XBP1s, for ChIP.

Figure 5. What is the expression of ASB11 in cell lines with P53 WT, P53 mutant (in your work MDA-MB157 and MDA-MB468) and P53 KO? Are the P53 KO cells similar to P53 mutant? Please discuss.

Ans: Although we did not investigate the impact of genotoxic agent on ASB11 expression in MDA-MB157 (p53 null) and MDA-MB468 (p53 R273H mutant) cells, we do provide data with HCT116 cells (p53 null) and H1299 cells (p53 R175H mutant). Our findings indicate that genotoxic agent slightly elevated, rather than reduced, the expression of ASB11 in both HCT116 (p53 null) and H1299 (p53 R175H mutant) cells (Fig. 5A and Fig. S3A). Furthermore, introduction of wild type p53 into both cell lines rescues genotoxic agent-induced ASB11 downregulation. Thus, as least for p53 R175H mutant, it behaves as a lost-of-function mutant in DNA damage-induced ASB11 expression. This is included in the Discussion section (p.20, the second paragraph).

Figure 5b. It's very interesting that in P53 KO cells the genotoxic stress increases the ASB11 expression in opposition to P53 wt cells, please discuss.

Ans: This is indeed an interesting phenomenon and is observed with both p53-null and p53-mutated cells. DNA damage even slightly elevates ASB11 level. Since there is no evidence for an activation of IRE1α pathway by DNA damage, it would require further study to dissect the underlying mechanism. We discuss this issue in the Discussion section (p.20, the second paragraph).

Figure 5c. The effects on BIK expression in shRNA ASB11 cells under genotoxic condition it's very subtle and similar to control experiments, please discuss.

Ans: The purpose of Fig. 5C is to study the impact of ASB11 on BIK protein turnover (half-life), rather than on BIK protein expression. To accurately compare the turnover of BIK in different conditions, we do not want to see huge differences in the intensities of BIK band at the initial time point (0 h). This is because band intensity on

Western blot does not follow a linear relation when the target of detection falls into very high or low intensity range. Therefore, we adjusted the exposure time of Western blot so that the BIK intensities at initial time point are comparable. This is now stated in the figure legend to avoid confusion.

Figure 7a. What is the statistical significance of this figure?
Ans: The statistic information is now included in Fig. 8A

Please discuss the effect of STF on the IRE1/XBP1 axis inhibition and the possible BIK stabilization and cellular apoptosis.
Ans: We include a sentence in the Discussion section that STF could in principle stabilize endogenous BIK via ASB11 downregulation, which may contribute to a small part of the tumor-killing effect of combined treatment (p.18, the second paragraph).

Finally in tumor cells, the inhibition of IRE1 activity and its consequences on BIK stability could be independent of P53 expression/status because the Hs578T cell lines using in the preclinical models have a point mutation in the P53 gene, please discuss.
Ans: We agree with the reviewer. In fact, since IRE1α acts downstream of p53, it is conceivable that p53 status would not affect the effect of combined treatment. We include it in the Discussion section (p.18, the second paragraph).

June 20, 2019

RE: JCB Manuscript #201901156R

Dr. Ruey-Hwa Chen
Academia Sinica
128 Academia Rd., Sec II, Nankang
Taipei 11529
Taiwan

Dear Dr. Chen:

Thank you for submitting your revised manuscript entitled "BIK ubiquitination controls life-death fate of cellular stress responses and anti-tumor activity". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

- Provide supplementary text as a separate, editable .doc or .docx file
- Provide main and supplementary figures as separate, editable files according to the instructions for authors on JCB's website, paying particular attention to the guidelines for preparing images and blots at sufficient resolution for imaging and screening
- Provide tables as excel files
- Methods are too brief - describe in sufficient detail for experiments to be repeatable
- Add MW markers to His panels in Fig 1A, 1F 1G (Myc panel) 4D, 4F, 4L, 5D, 7D, S1C, S1E, S2H, S5A, S5B
- Suggested alternative title to make the main advance accessible to as broad an audience as possible:
"BIK ubiquitination by the E3 ligase ASB11 determines cell fate during cellular stress"
suggested edits to the abstract for clarity:
"The BH3-only pro-apoptotic protein BIK is regulated by ubiquitin-proteasome system. However, the underlying mechanism of this regulation and its physiological functions remain elusive. Here, we identify CRL5-ASB11 as the E3 ligase targeting BIK for ubiquitination and degradation. ER stress leads to the activation of ASB11 by XBP1s during the adaptive phase of the unfolded protein response, which stimulates BIK ubiquitination, interaction with p97/VCP, and proteolysis. This mechanism of BIK degradation contributes to ER stress adaptation by promoting cell survival. Conversely, genotoxic agents downregulate this IRE1□/XBP1s/ASB11 axis and stabilize BIK, which contributes in part to the apoptotic response to DNA damage. We show that blockade of this BIK degradation pathway by an IRE1□ inhibitor can stabilize a BIK active mutant and increase its anti-tumor activity. Our study reveals that different cellular stresses regulate BIK ubiquitination by ASB11 in opposing directions, which determines whether or not cells survive, and that blocking BIK degradation has the potential to be used as an anti-cancer strategy."

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A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

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Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Nika Danial, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The authors have addressed all my prior concerns with new experimental data that supports their conclusions. As such, the manuscript is even stronger.

Reviewer #2 (Comments to the Authors (Required)):

The authors addressed all the questions requested by the reviewer very well. I think the manuscript is convincing.
One very minor point is about the molecular weight marker on the immunoblots. Some of the blots are missing the marker, which is highly recommended to add.