The CRL4 E3 ligase Mahjong/DCAF1 controls cell competition through the transcription factor Xrp1, independently of polarity genes

Amit Kumar and Nicholas E. Baker
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Original submission

First decision letter

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MS TITLE: The CRL4 E3 ligase Mahjong/DCAF1 controls cell competition through the transcription factor Xrp1, independently of polarity genes

AUTHORS: Amit Kumar and nicholas e baker

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee's comments, and we will look over this and provide further guidance.

Reviewer 1

Advance summary and potential significance to field

In this manuscript, Amit Kumar and Nicholas Baker describe a new function of the transcription factor Xrp1 in the regulation of the loser status of the Mahjong mutant cells. Previously, Mahjong mutant were described as characteristic loser cells which are eliminated by apoptosis upon JNK activation when surrounded by WT cells. More importantly, Mahjong was epistatically associated with defects in apico-basal polarity and the Lgl mutant (which are also eliminated by apoptosis) due to physical interaction, and the rescue of lgl mutant elimination by Mahjong overpexression (Tamori et al. 2010). Since then, Mahjong background was considered as a good proxy for cell competition associated with polarity genes mutant. Recently, similar transcriptional signatures were found in the Minute mutant cells (the historical model of cell competition), and in Mahjong

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mutant (including oxidative stress and proteotoxic stress), suggesting that these signatures were universal hallmarks of cell competition (either related to Minute, Myc or to polarity mutants) and key determinant of loser status. This goes back to the central debate about the existence of a unique signature of loser status and cell competition and the uniqueness of mechanism driving cell elimination, or whether there are clear distinctive features depending on the type of mutations.
By clearly showing that the elimination of Mahjong mutant is not related to polarity genes, but rather by the induction of the transcription factor Xrp1, related to the ubiquitin ligase activity of Mahjong, and by showing on the contrary that Scribble and Lgl mutant clones elimination does not rely on Xrp1, this article argues strongly for a clear distinction between polarity mutant elimination compared to Minute and Mahjong. I believe this is a very important point that fully justify publication in Development.
While it is true that a recent publication already showed that Xrp1 is required for Mahjong mutant elimination (Langton et al 2021), this manuscript went through much deeper characterization of the mechanism (where there are admittedly still big unknowns)
and made this clear distinction with polarity mutant which is an essential point. The demonstration is convincing (although some results may deserve a few more samples/observations), I only have a few suggestions that would help to definitely clarify the link between Mahjong and Lgl.

Comments for the author

1. The authors propose an explanation in the discussion for the rescue of Lgl mutant by Mahjong overpexression, which through the downregulation of Hippo pathway could prevent Lgl mutant clone elimination. Could the author check whether the Hippo pathway is indeed downregulated upon overpexression of Mahjong in clone or in a compartment (using one of the transcriptional reporter of Yki targets, ex-lacZ, diap1-lacZ…) ?
2. For many results, the number of samples seems really low (n=2 or n=4)
which questions the robustness of the results, specially if there is some inter disc variability. I do acknowledge that perturbation in clones or in one compartment provide a nice internal control, but it may still deserve a few more observations to be fully conclusive. This is even more essential for observation made throughout the disc (e.g: : lysotracker in Figure 2C).
3. Since the absence of requirement of Xrp1 in lgl and scribble mutant elimination is a central point of this manuscript, I think it would be good to provide some quantification for the dcp1 staining to compare the number of apoptotic cells in lgl-/- vs lgl-/- Xrp1-/- and scrib-/- vs Scrib-/- Xrp1-/-.

Other minor points :
- Line 172-174 and Line 97 98: the authors state that autophagy was reported to be elevated in Nagata et al 2019, and also Baumgartner et al 2021.
If I am correct these two studies had contradictory results on these aspects and the results in Baumgartner et al rather suggested that autophagy is impaired in Mahjong mutant (notably with the p62 pulse chase experiment). This should be corrected in the text, or at least described a bit more precisely to be totally correct.
- For some graphs, it is not always clear what represent single dots (clone or wing disc ?), specially when counting cell death per unit of perimeter. Could the authors clearly specify that in the legends ?
- Line 155: the sentence may need to be reformulated. I guess the authors meant the within an homogeneous Majhong mutant background, Xrp1+ cells are outcompeted by Xrp1- cells ? (all being Mahjong mutant). The sentence in its current formulation was a bit confusing.

Reviewer 2

Advance summary and potential significance to field

Understanding whether and how organisms specifically eliminate slow growing cells in normally growing tissue is of key importance in developmental and cancer biology. Drosophila research has provided the first description of this process known as cell competition, and has been instrumental to elucidate molecular players.
The authors provide important details on how activation of the Xrp1 transcription factor is achieved in CRL4 E3 ligase Mahjong (Mahj) mutant clones, known to be eliminated by cell competition.

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Together with a previous study (Langton et al 2021), they demonstrate that Xrp1 is required for mahj clones elimination, and provide further information by describing how Xrp1 is responsible for many of the "loser" status cellular hallmarks (elevated autophagy, JNK pathway activation and reduced protein synthesis).
The authors highlight that the mechanism of Xrp1 induction in mahj mutant clones differs from the one operating in Minute (Rp/+) mutant clones, which activates Xrp1 in an RpS12-dependent manner. This fact contrast with the remarkable similarity of the cellular response in mahj and Minute clones, pointing to the induction of Xrp as the key factor, irrespective of the mechanism of activation.
In contrast, the authors show that in yet another cell competition model, the lgl and scribl mutant clones previously thought to be eliminated by a Mahj-dependent mechanism, Xrp1 is not induced nor required. The authors note that the relevance of the reported physical interaction between Lgl and Mahj remains to be determined.
Overall, the present work provides solid evidence for a specific Xrp1 induction mechanism in mahj clones, that contrasts with the ones known to date. This conclusion contributes to the concept of Xrp1 as a stress-responsive transcription factor important for the elimination of the stressed cells, independently of the triggering factor. The above mentioned diversity in the requirement for Xrp1 in cell competition is also important in the classification of the different cell competition paradigms in Drosophila.
The work is suitable for publication after addressing the following points:

Comments for the author

Major points:
- Since mahj dowregulation does not affect expression of the Xrp1-LacZ transcriptional reporter (Supplementary Figure 3G-G'), it is important to pay special attention to the usage of the term "Xrp1 expression". This term, which normally refers to gene transcription, is frequently used in the text to refer to Xrp1-HA protein levels, and is therefore confusing. Line 263, it is stated that "This strongly suggests that mahj regulates cell competition through CRL4-dependent ubiquitination of a protein that would otherwise promote Xrp1 expression". Here, "Xrp1 expression" should be replaced by "Xrp1 protein levels", "Xrp1-stability" or similar, to emphasize the fact that target protein X (Figure 7) does not affect Xrp1 transcription, but rather translation or protein stability. In this regard, it is key to confirm the data obtained using the Xrp1-LacZ transcriptional reporter (Supplementary Figure 3G-G') by qRT-PCR expression data on Xrp1 in discs under mahj downregulation.
-Since most of the work is related to Xrp1 regulation in mahj clones, it is important to fully exclude the possibility that Mahj might regulate Xrp1 protein levels through Warts. For this purpose, it is necessary to show that Warts loss-of-function does not rescue the increase in Xrp1-HA levels in a Mahj loss-of-function background (similarly to what is shown in Figure G-H' using en-Gal4, UAS-GFP > dsRNAmahj, UAS-Yki), to exclude that Warts controls Xrp1 protein levels in a Yorkie-independent manner. An experiment showing that Warts overexpression does not increase Xrp1-HA levels (similarly to what is shown in Figure E-F' with nub-Gal4, UAS-GFP > UAS-Hippo) would also help to make this conclusion more robust and avoid indirect testing using Hippo over-expression.
-Statistical analysis: it is specified in Materials and Methods that "Statistical comparisons were made using t-test assuming normal distribution". The assumption of normal distribution, critical for the application of T-tests, is not appropriate, especially in cases of the low N numbers indicated throughout the work. Tests for normal distribution have low potency with low N numbers and will very frequently result in false positive corroborations of normal distributions.
In this case it is appropriate to use a non-parametric T-test, usually available in statistical analysis software, to test statistical differences without assuming normal distribution.

Minor points:
-Line 127: The size of the posterior compartment is said to be reduced "with respect to the total size of the wing disc", while in the referred Supplementary Figure 1C the anterior to posterior compartment size ratio is shown.
-Line 505: Correct phrase: "especially in the wing pouch which is more common in clones of pouch domain".
-Figure 1G: the y-axis label "cell death per unit clone perimeter" is not properly described in the figure legends, nor in Materials and Methods. How is the clone perimeter defined?
-Line 521: the word "black" should be "white"?

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-Figures 1K-O: Specify the Gal4 driver line used.
-Supplementary Figure 1 D-F': Provide quantification of cell death per unit clone perimeter, as shown in Figure 1G.
-Figure 2: Provide quantification for the three parameters shown: level of pJNK in GFP-positive clones, Lysotracker spots/label per wing disc, OPP levels in GFP-
positive clones. Circumscribe the quantifications to wing pouch if necessary.
-Figure 3: Similarly, please provide quantification of the Xrp1-HA signal in the relevant compartment or clones, for each experiment.
-Supplementary Figure 3: Replace "ß-Gal" label by the name of the reporter being analyzed (for example, "rpr-LacZ".
-Figure 4C: the "dsRNACul4KD" label should be replaced to better indicate that a dominant negative form of the protein is being over-expressed.
-Figure 5D: the y-axis label "cell death per unit clone length" is not properly described in the figure legends, nor in Materials and Methods. Is this quantification similar to that in Figure 1G, in which "cell death per unit clone perimeter" is indicated?
-Figure 5I: The panel is not visible in the PDF file used for review.

Reviewer 3

Advance summary and potential significance to field

This is an interesting paper in which the authors examine the mechanism by which cell competition is induced in cells lacking Mahj, a CRL4 ubiquitin ligase. Mahj acts as a substrate receptor protein for Cullin 4 and DDB1, targeting Warts and other proteins for ubiquitylation and proteosomal degradation. mahj mutant cells are known to be competed against in mosaic tissues and have been analyzed at the transcriptional level, where they show similar gene expression profiles as some Rp/+ mutant cells. For example, both lead to activation of various stress pathways and induce the stress response activator, Xrp1. The transcriptional similarities and the fact that cells of each mutant are out-competed in mosaics has led to the idea that they are part of a "signature" of loser cells, and the Piddini lab proposed that they are competitively eliminated by a common mechanism. Here the authors show that as in Rp/+, Xrp1 is involved in the elimination of mahj mutant cells in mosaics. However, Xrp1 is not induced by any of the known mechanisms (p53, in response to irradiation, and RpS12, in response to Rp heterozygosity). They carry out several experiments in which they manipulate levels of cull4 and ddb1/pic, that lead them to propose that Xrp1 may be induced by an as yet unidentified protein that is normally degraded in a Mahj-dependent way. As a known substrate for Mahj, Warts was tested by the authors as a candidate for such a protein, but found not to be the case. Interestingly, despite the known physical association between Mahj and Lgl and the previous report that expression of Mahj in lgl mutant clones can prevent their elimination, the authors find that the competitive loss of cells deficient for these genes is mechanistically different, as the loss of polarity genes (lgl, scrib) does not induce Xrp1 and they are still eliminated from mosaic tissues in an Xrp1 mutant background. Overall, the data support a model in which Xrp1 functions as a general sensor of cellular defects that can lead to cell competition, and can be induced by a variety of stresses such as proteotoxicity, reduced translation, and shown here, altered ubiquitylation. The paper is written clearly, the experiments are well done and the data is convincing (although N values are very low, see below), and the work provides an important and significant advance to our knowledge about mechanisms of cell competition.

Comments for the author

For all figures, the N values are very small: often 4 (ie, 2 larvae), sometimes 8 or 9 (ie, 4 or 5 larvae scored) per experiment. It is not clear what N refers to, although in the Methods, it is stated, "Number of wing discs studied, reported as N values in figure legends, represent biological replicates". This makes things even more confusing. Please define the N more clearly, and how they represent biological replicates? Related to this concern, the authors consistently use SEM as a measurement of variation in their graphs. Unless the data is from several independent biological experiments with high Ns, the authors should show the standard deviation, not the standard error of the mean.

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The authors state in line 138 that in Fig. 1C, cell death is seen at the boundaries of mahj mutant cells and wildtype cells. However, the wildtype cells are not distinguished from the mahj+/- cells in the image, as the twin spots are not labelled. The authors should correct the statement accordingly.
In Fig2 B", why is the p-JNK background so high? The image quality in Fig. 2C-E is also very low and the lysotracker is quite hard to see.
In the text describing data in Figure S3 (lines 212-214), panel H is described before panel G. Match the text to the figure?
In Fig 3S, please define the Gal4 driver in D-E, and show where the P compartment is. Also, could the authors show the data for the controls in L, since they mention it in the text?
Presumably Cul4 and ddb1 are used in many processes in disc development, yet it is interesting that knockdown of the Cul4 neddylation mutant is much more severe than that of the intact Cul4 version (Fig 4C). Also knockdown of the proteosome component is much more severe. Does this suggest that in these cases Xrp1 fucntions with some other factor?
In Fig. 5, panel I, "Quantification of ddb1 knock-down clones shown in panels G-I", is missing.
Also Fig. 5, should the label "p-eIF2a" in J be in magenta, to match the color in the figure?
And, why does the effect of cul4 RNAi shown in 5J, J' look so much more severe than in Fig 4A, where nubbin-Gal4 and cul4-RNAi was also used?
It's interesting that there are so many Dcp1-positive cells within clones of cul4-RNAi and dbb1 KD, as well as on the clone borders in Fig. 5B'. Does this really differ from what is normally observed in discs with Rp/+ cells or Mahj KD clones? If so, could the authors speculate why this is the case?
In the legend to Fig. 7C, please clarify what driver is used to express the Xrp1 RNAi in the lgl mutant clones.
Line 676: Do the authors intend to use the word "presumptively" or is this a mis-spelling of "presumably"?

First revision

<u>Author response to reviewers' comments</u>

Reviewer 1 Comments for the Author: 1.The authors propose an explanation in the discussion for the rescue of Lgl mutant by Mahjong overpexression, which through the downregulation of Hippo pathway could prevent Lgl mutant clone elimination. Could the author check whether the Hippo pathway is indeed downregulated upon overpexression of Mahjong in clone or in a compartment (using one of the transcriptional reporter of Yki targets, ex-lacZ, diap1-lacZ…) ?

***Reply**: We appreciate the experiment suggested by the reviewer. We have done this experiment and shown the results in Figure S7A,B. Since we did not see changes in Yki reporter lines after mahj over-expression, we changed our speculation about Mahj and Lgl interaction accordingly (please see lines 377-381).*

2. For many results, the number of samples seems really low (n=2 or n=4) which questions the robustness of the results, specially if there is some interdisc variability. I do acknowledge that perturbation in clones or in one compartment provide a nice internal control, but it may still deserve a few more observations to be fully conclusive. This is even more essential for observation made throughout the disc (e.g: : lysotracker in Figure 2C).

***Reply**: We recognize reviewer concerns and repeated many experiments, including the one with lysotracker (Figure 2C). The increased n are listed in the Figure legends. None of our results were changed by the increased sample numbers.*

3. Since the absence of requirement of Xrp1 in lgl and scribble mutant elimination is a central point of this manuscript, I think it would be good to provide some quantification for the dcp1 staining to compare the number of apoptotic cells in lgl-/- vs lgl-/- Xrp1-/- and scrib-/- vs Scrib-/- Xrp1-/-.

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***Reply**: Quantification of competitive cell death in lgl and scrib mutant clones is now shown as supplementary figure S8 A & B, respectively.*

Other minor points :
-Line 172-174 and Line 97 98: the authors state that autophagy was reported to be elevated in Nagata et al 2019, and also Baumgartner et al 2021. If I am correct these two studies had contradictory results on these aspects, and the results in Baumgartner et al rather suggested that autophagy is impaired in Mahjong mutant (notably with the p62 pulse chase experiment).

This should be corrected in the text, or at least described a bit more precisely to be totally correct.

***Reply**: The reviewer is not quite correct. Both groups found increased autophagosome accumulation, which Baumgartner et al showed was due to reduced autophagic flux. This did not contradict the data of Nagata et al, who did not measure flux, although it did differ from their interpretation. We have rewritten our paper to refer more accurately to the observations of autophagosome accumulation in mahj mutants, and to mention the different interpretations, please see lines 170-175.*

-For some graphs, it is not always clear what represent single dots (clone or wing disc ?), specially when counting cell death per unit of perimeter. Could the authors clearly specify that in the legends ?

***Reply**: This information has been added to all the figure legends.*

-Line 155: the sentence may need to be reformulated. I guess the authors meant the within an homogeneous Majhong mutant background, Xrp1+ cells are outcompeted by Xrp1- cells ? (all being Mahjong mutant). The sentence in its current formulation was a bit confusing.

***Reply**: We clarified this sentence as requested. See line 147-148 for these changes.*

Reviewer 2

Reviewer 2 Comments for the Author:

Major points:
- Since mahj dowregulation does not affect expression of the Xrp1-LacZ transcriptional reporter (Supplementary Figure 3G-G'), it is important to pay special attention to the usage of the term "Xrp1 expression". This term, which normally refers to gene transcription, is frequently used in the text to refer to Xrp1-HA protein levels, and is therefore confusing. Line 263, it is stated that "This strongly suggests that mahj regulates cell competition through CRL4-dependent ubiquitination of a protein that would otherwise promote Xrp1 expression". Here, "Xrp1 expression" should be replaced by "Xrp1 protein levels", "Xrp1-stability" or similar, to emphasize the fact that target protein X (Figure 7) does not affect Xrp1 transcription, but rather translation or protein stability. In this regard, it is key to confirm the data obtained using the Xrp1-LacZ transcriptional reporter (Supplementary Figure 3G-G') by qRT-PCR expression data on Xrp1 in discs under mahj downregulation.

***Reply**: We replaced 'expression' with 'protein expression' wherever appropriate in the manuscript. It was not our intention to imply that Xrp1 transcription is not changed. In fact it is previously established that Xrp1 transcription is elevated in mahj mutants (Kucinski et al., 2017) and we now explain this explicitly. Actually, this was the basis for initiating this project and is the first sentence of the Results section. Since we found that an Xrp1 enhancer trap is not elevated, we just thought it worth mentioning that this may be an imperfect reporter for Xrp1 transcription, which is also our conclusion from other studies in our lab. We did not intend to imply any discrepancy between transcription and translation of Xrp1, and have clarified the manuscript to make this clear (please see lines 211-215).*

-Since most of the work is related to Xrp1 regulation in mahj clones, it is important to fully exclude the possibility that Mahj might regulate Xrp1 protein levels through Warts. For this

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purpose, it is necessary to show that Warts loss-of-function does not rescue the increase in Xrp1-HA levels in a Mahj loss-of-function background (similarly to what is shown in Figure G-H' using en-Gal4, UAS-GFP > dsRNAmahj, UAS-Yki), to exclude that Warts controls Xrp1 protein levels in a Yorkie-independent manner. An experiment showing that Warts overexpression does not increase Xrp1-HA levels (similarly to what is shown in Figure E-F' with nub-Gal4, UAS-GFP > UAS-Hippo) would also help to make this conclusion more robust, and avoid indirect testing using Hippo over-expression.

Reply: *The point raised by reviewer is important and we added these experiments during the revision, although we used Warts RNAi not Warts mutants, because of the locations of warts and mahj on different chromosomes, as well as the Warts over-expression experiment. The new results, which were similar to those already shown for Hippo and Yorkie, are included in the revised Figures 6E-H, S7I. The Hippo and Yorkie data included previously is not moved to supplemental figures (S7E-F). This new data indeed supports in a more direct manner our conclusion that Xrp1 expression in mahj mutant cells is independent of wts.*

-Statistical analysis: it is specified in Materials and Methods that "Statistical comparisons were made using t-test assuming normal distribution". The assumption of normal distribution, critical for the application of T-tests, is not appropriate, especially in cases of the low N numbers indicated throughout the work. Tests for normal distribution have low potency with low N numbers and will very frequently result in false positive corroborations of normal distributions. In this case it is appropriate to use a non-parametric T-test, usually available in statistical analysis software, to test statistical differences without assuming normal distribution.

Reply: *We addressed this point by replacing t-tests with the non-parametric Mann-Whitney test whenever n<10 or where normality was otherwise questionable. Not differences in the significance of any conclusions resulted. The statistical procedures are described in lines 486-491 of the revised manuscript.*

Minor points:
-Line 127: The size of the posterior compartment is said to be reduced "with respect to the total size of the wing disc", while in the referred Supplementary Figure 1C the anterior to posterior compartment size ratio is shown.

Reply: *Thank you for pointing this out. The results section was changed to match the Figure legend.*

-Line 505: Correct phrase: "especially in the wing pouch which is more common in clones of pouch domain".

Reply: *This was corrected (lines 512-513)*

-Figure 1G: the y-axis label "cell death per unit clone perimeter" is not properly described in the figure legends, nor in Materials and Methods. How is the clone perimeter defined?

Reply: *This is now clearly explained in material-methods, see line 478-479*

-Line 521: the word "black" should be "white"?

Reply: *Corrected.*

-Figures 1K-O: Specify the Gal4 driver line used.
Reply: *The 109-68 Gal4 driver is now described in the figure legend (lines 529-530).*

-Supplementary Figure 1 D-F': Provide quantification of cell death per unit clone perimeter, as shown in Figure 1G.

Reply: This quantification is now provided as Fig S1H.

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-Figure 2: Provide quantification for the three parameters shown: level of pJNK in GFP-positive clones, Lysotracker spots/label per wing disc, OPP levels in GFP- positive clones. Circumscribe the quantifications to wing pouch if necessary.

Reply: Quantification is now provided for pJNK and lysotracker in Supplementary Figure S2, as requested. We did not quantify average OPP levels because translation is patch in wing discs (Lee et al., 2018) and it is better to compare nearby cells inside and outside clones.

-Figure 3: Similarly, please provide quantification of the Xrp1-HA signal in the relevant compartment or clones, for each experiment.

Reply: Quantification of Xrp1-HA is now included as requested Fig S3A, S4A, Fig4H.

-Supplementary Figure 3: Replace “ß-Gal” label by the name of the reporter being analyzed (for example, “rpr-LacZ”.

***Reply**: Corrected.*

-Figure 4C: the “dsRNACul4KD” label should be replaced to better indicate that a dominant negative form of the protein is being over-expressed.

***Reply**: dsRNACul4KD was replaced with UAS-Flag-Cul4KR in Fig 4C*

-Figure 5D: the y-axis label “cell death per unit clone length” is not properly described in the figure legends, nor in Materials and Methods. Is this quantification similar to that in Figure 1G, in which “cell death per unit clone perimeter” is indicated?

***Reply**: Yes, both are similar and the same description is now used in the revised manuscript*

-Figure 5I: The panel is not visible in the PDF file used for review.
Reply: this panel was added to the revised Figure.

Reviewer 3 Advance Summary and Potential Significance to Field:

Reviewer 3 Comments for the Author:
For all figures, the N values are very small: often 4 (ie, 2 larvae), sometimes 8 or 9 (ie, 4 or 5 larvae scored) per experiment. It is not clear what N refers to, although in the Methods, it is stated, “Number of wing discs studied, reported as N values in figure legends, represent biological replicates”. This makes things even more confusing. Please define the N more clearly, and how they represent biological replicates? Related to this concern, the authors consistently use SEM as a measurement of variation in their graphs. Unless the data is from several independent biological experiments with high Ns, the authors should show the standard deviation, not the standard error of the mean.

Reply: The description in the Methods was revised to clearly define n values, please see line 481. Alll graphs now show Standard Deviation as error bars.

The authors state in line 138 that in Fig. 1C, cell death is seen at the boundaries of mahj mutant cells and wildtype cells. However, the wildtype cells are not distinguished from the mahj+/- cells in the image, as the twin spots are not labelled. The authors should correct the statement accordingly

***Reply**: This correction was made as requested, please see lines 116-118.*

In Fig2 B”, why is the p-JNK background so high? The image quality in Fig. 2C-E is also very low and the lysotracker is quite hard to see.

Reply: Parts of the peripodial membrane were included by mistake. This image was reprepared excluding sections from the peripodial membrane.

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In the text describing data in Figure S3 (lines 212-214), panel H is described before panel G. Match the text to the figure?

Reply: corrected as requested. See lines 211-215.

In Fig 3S, please define the Gal4 driver in D-E, and show where the P compartment is. Also, could the authors show the data for the controls in L, since they mention it in the text?

***Reply**: Supplementary 3 in previous version is now divided into two figures: Supplementary 3 & The tub-Gal4 driver is now described in the figure legends. Because tub-Gal4 is not compartment-specific, the position of the P compartment is not relevant. The previous Figure S3L is now S4G. S4G is the quantification of results shown in S4F. The anterior compartments are the controls for the posterior compartments, there is no other control. Hopefully this will be clear in the revised manuscript.*

Presumably Cul4 and ddb1 are used in many processes in disc development, yet it is interesting that knockdown of the Cul4 neddylation mutant is much more severe than that of the intact Cul4 version (Fig 4C). Also knockdown of the proteosome component is much more severe. Does this suggest that in these cases Xrp1 fucntions with some other factor?

***Reply**: As these experiments were not performed in parallel, it may not be appropriate to compare the results quantitatively. The differences in wing pouch size may also indicate that Cul4 and proteasomes regulate other targets besides Xrp1*

In Fig. 5, panel I, “Quantification of ddb1 knock-down clones shown in panels G-I”, is missing.

***Reply**: Panel I was added to the revised figure*

Also Fig. 5, should the label “p-eIF2a” in J be in magenta, to match the color in the figure?
And, why does the effect of cul4 RNAi shown in 5J, J’ look so much more severe than in Fig 4A, where nubbin-Gal4 and cul4-RNAi was also used?

***Reply**: p-eIF2A labeling color is now in Magenta.*

As these experiments were not performed in parallel, there may have been small differences in, for example, culture temperature or dissection time that could impact the results quantitatively. It would be risky to draw conclusions from quantitative comparisons except for experiments performed in parallel.

It’s interesting that there are so many Dcp1-positive cells within clones of cul4-RNAi and dbb1 KD, as well as on the clone borders in Fig. 5B’. Does this really differ from what is normally observed in discs with Rp/+ cells or Mahj KD clones? If so, could the authors speculate why this is the case?
***Reply**: We believe it is different from Rp/+ or mahj clones. The explanation may be that cul4 and dbb1 contribute to viability through additional mechanisms. Please see lines 265-266.*

In the legend to Fig. 7C, please clarify what driver is used to express the Xrp1 RNAi in the lgl mutant clones.
***Reply**: tub-Gal4 of MARCM FRT40 stock, mentioned in revised figure*

Line 676: Do the authors intend to use the word “presumptively” or is this a mis-spelling of “presumably”?
***Reply**: This was a mis-spelling, now corrected. Please see line 626.*

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Second decision letter

MS ID#: DEVELOP/2022/200795

MS TITLE: The CRL4 E3 ligase Mahjong/DCAF1 controls cell competition through the transcription factor Xrp1, independently of polarity genes

AUTHORS: Amit Kumar and nicholas e baker
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The authors have adressed all my concernes. I fully support publication of the manuscript in its current form. I only have one very last suggestion of text editing (just adding two words, see below)

Comments for the author

Line 337 in the discussion the authors state that "Mahj regulates Xrp1 through its ubiquitin ligase activity". While the fact that DDB1 Cul4 and Roc1A KD phenocopy Mahj mutant is a good argument, definitely proving this point would require to find a mutant of Mahj specifically affecting its ubiquitin ligase activity and recapitulating Xrp1 induction. I would therefore suggest to be less assertive and replace this sentence by "Mahj regulates Xrp1 most likely through its ubiquitin ligase activity".

Reviewer 3

Advance summary and potential significance to field

See previous review

Comments for the author

I am fine with the paper now and support publication. Sorry for the delay.

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