Phosphorylation of the exchange factor DENND3 by ULK in response to starvation activates Rab12 and induces autophagy

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Review timeline:

Submission date: 15 December 2014
Editorial Decision: 12 January 2015
Revision received: 12 March 2015
Editorial Decision: 27 March 2015
Revision received: 30 March 2015
Accepted: 31 March 2015

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.)

Editor: Nonia Pariente

1st Editorial Decision 12 January 2015

Thank you for your patience during the peer-review of your study at EMBO reports. We have now received the three enclosed referee reports. As you will see, all the referees find the topic of interest, and referees 1 and 3 are rather positive, although referee 2 raises a number of concerns.

Given that all referees provide constructive suggestions on how to strengthen the work, I would like to give you the opportunity to revise your manuscript. In this case, the concerns of referee 1, which are also echoed by referee 2, should be addressed, as well as points 2-4 of referee 3. In addition, referee 2 raises several important concerns regarding the conclusiveness of the data, and all of these need to be addressed in full. However, a number of other points deal with additional mechanistic insight and, although it would be preferable to address them at least to some extent, this would not be a precondition for publication (these include mapping the interaction sites of ULK1 and DENND3, and points 4 and 5, as well as referee 3’s point 1).

If the referee concerns can be adequately addressed, we would be happy to consider your manuscript for publication in EMBO reports. However, please note that it is our policy to undergo one round of revision only and, thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

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I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, please contact me if I can be of any assistance.

REFEREE REPORTS:

Referee #1:

This study reports that DENND3 positively regulates starvation induced autophagy. The authors show that this is likely mediated via Rab12, which localises to autophagosomes. The DENND3-Rab12 cascade is regulated by ULK phosphorylation in a starvation-dependent manner.

Overall, the paper makes very plausible connections that link a number of areas pertinent to autophagosome biogenesis. For the most part, the data are convincing. It is clear that DENND is phosphorylated by ULK proteins at S572 and S554 in a starvation-dependent manner. The nice experiments in Fig3 show that this event regulates the activation of RAB12. While the exact consequences of this for autophagosome biogenesis etc. are not explored in great depth, the localisation data are clear as are the effects on autophagosome movement.

For me, the one area that can be neatened is Fig 1 and S1. While Fig 1A and B are convincing, I suggest that the blots could be improved. First the authors should compare LC3-II with GAPDH as outlined in the Klionsky et al guidelines. Second, S1 should be done with both oligos - the CQ data are important as they enable inference of the autophagosome formation rate.

At face value the effects described appear to be obvious in starvation but less clear in basal conditions. However, the data in 1A/B suggest that the effects do exist in unstarved cells and maybe the clearer effects in the starved cells simply reflect the different floor effects of the GFP-LC3 dot counting versus western blotting assays. I wondered whether some comment on this would be helpful in the discussion.

Referee #2:

Xu and colleagues describe a regulation of autophagy through Rab12, activated after starvation by DENND3 which they show is a substrate of overexpressed ULKs. They identified the phosphorylation site on DENND3 as binding site for 14-3-3- proteins by mass spec in DENND3 immunoprecipitations. Starvation increases the phosphorylation of DENND3, and activation of Rab12. Finally they show recombinant or overexpressed Rab12 binds LC3.

Previous reports in the literature regarding Rab12, and DENND3, as well as a report that DENND3 binds LC3 all support the authors theory that activated Rab12 may regulate autophagy. However, it is not clear how Rab12 functions, and the data is on the whole preliminary and requires additional experiments both for validation of the existing data andf to uncover the mechanism of action of activated Rab12.

Major points:
1. Figure 1, the formation of GFP-LC3 puncta is clearly influenced by loss of DENND3. On the other hand the endogenous LC3 lipidadtion and p62 are not conclusive. LC3-lipidation is typically expressed as LC3-II over a housekeeping protein. From the western blots shown LC3-II levels do not look substantially changed and the difference may be due to changes in LC3-I levels. In addition, while p62 degradation does appear to be inhibited by loss of DENND3 in starvation, it is surprising that in the unstarved DENND3 siRNA sample that p62 has not accumulated. Figure S1 uses chloroquine to inhibit lysosome. See recent paper (Florey et al., 2014 Autophagy) as this is not recommended now as CQ has been shown to induce LC3-lipidation.
2. In Figure 1C, 2C, D, S3A,B,C and 2F the authors show phosphor analyisi with anti-pS554. From the CIP data in Fig. S2B this antibody is clearly not phosphor-specific and is not useful. All these panels require normalization to the total DENND3 protein as the protein levels themselves might be altered. In addition the changes in pS554 are minor in Fig 2D considering the interaction with the non-phospho protein.
3. The authors report the ULK has been shown to bind their kinase substrates. The binding they refer to is the interaction of the c-terminal region of ULK1 with Atg13. This binding has not been shown to regulate the phosphorylation of Atg13. To demonstrate direct binding in Fig S3D, E the authors should show using endogenous proteins the interaction of ULK1 and DENND3, and map the binding sites on both proteins. This could be used to look at mutants of DENND3 that can't bind ULK.

4. The rescue experiment shows that each phosphosite alone is required for rescue. This is interesting and has implications for activation, and 14-3-3 binding. This would mean that the phosphor-mimic protein would have to be a S554DS572D (or E mutants) to rescue like wt.

4. The authors show in Fig. 3 RILP1 binds activated Rab12. In Fig. 4 A ands B in the presence of GTPgammaS would they predict RIPL1 would be bound? and could this compete for LC3 interaction?

5. Where do Rab12 and LC3 interact? Is it via a LIR motif in LC3? Is Rab12 a cargo degraded in autophagosomes?

6. Fig. 4 panel D the LC3 would presumably be on membranes if co-localizing with Rab12. An additional informative measure would also include the % of LC3 which was Rab12 negative- this should change in starvation.

7. Fig. 4C. There seems to be a large amount of LC3 in the cells overexpressing Rab12. This might indicate that Rab12 actually inhibits maturation of LC3-positive autophagosomes. Flux analysis with BafA should be done in these cells (using conditions which give higher transfection efficiency).

Minor

1. A GST control is required in Fig. S4B.

2. Are the plasmids used in Fig. 3B siRNA-resistant? If so please supply information.

Referee #3:

This manuscript is very clearly written, and presents high quality data supporting an intriguing model linking starvation to autophagosome formation and autophagy, using beautiful and convincing biochemistry and cell biology approaches. I don't think any additional experiments are necessary for this manuscript to be impactful, interesting and compelling; however, if the authors have in hand some of the results I ask about below, or could add in some text to address some of these questions, it would strengthen this manuscript if they were included.

1. Have starvation times longer than 120 min been applied? I wonder if this signaling pathway is transiently activated, vs. generating a sustained signal that is not down regulated until the stimulus is removed.

2. ULK is described as homologous to the yeast kinase Atg1. Are there other known parallels of the signaling pathway uncovered here with yeast proteins/interactions/pathways?

3. Does siRNA of any of the 14-3-3 proteins also affect autophagy? I realize this may be too difficult to do if there is too much redundancy in the 14-3-3 family and their expression in the cells used.

4. Do the authors have any ideas about how DENND3 and/or Rab12 might lead to activation of the motor proteins that are apparently necessary for mobility that facilitates completion of autophagy? This point in particular should be fleshed out a bit more in the text.
Detailed response to referees

Referee #1

Comment 1) This study reports that DENND3 positively regulates starvation induced autophagy. The authors show that this is likely mediated via Rab12, which localises to autophagosomes. The DENND3-Rab12 cascade is regulated by ULK phosphorylation in a starvation-dependent manner.

Overall, the paper makes very plausible connections that link a number of areas pertinent to autophagosome biogenesis. For the most part, the data are convincing. It is clear that DENND is phosphorylated by ULK proteins at S572 and S554 in a starvation-dependent manner. The nice experiments in Fig3 show that this event regulates the activation of RAB12. While the exact consequences of this for autophagosome biogenesis etc. are not explored in great depth, the localisation data are clear as are the effects on autophagosome movement.

For me, the one area that can be neated is Fig 1 and S1. While Fig 1A and B are convincing, I suggest that the blots could be improved. First the authors should compare LC3-II with GAPDH as outlined in the Klionsky et al guidelines. Second, S1 should be done with both oligos - the CQ data are important as they enable inference of the autophagosome formation rate.

Response to comment 1) First, we wish to thank the referee for the positive comments about our study. We agree with the referee that figure 1C and supplemental figure 1 need improvement, a comment also made by referee 2. As suggested, we repeated the experiments in figure 1C and supplemental figure 1 and have improved the quality of the blots. We also quantified the blots by normalizing the level of LC3-II to GAPDH as suggested (Fig. 1C). The experiments in figure S1 were also repeated using two different siRNAs. Comment 3 of Referee 2 suggested that we not use QC in supplemental figure 1 as it can induce LC3 lipidation. In comment 10, referee 2 indicated that we should use Bafilomycin A1 instead. Thus, we replaced the QC experiments in supplemental figure 1 with experiments performed in the presence of Bafilomycin A1. Finally, we incorporated all of this data into a new figure 1C and deleted the original supplemental figure 1.

Comment 2) At face value the effects described appear to be obvious in starvation but less clear in basal conditions. However, the data in 1A/B suggest that the effects do exist in unstarved cells and maybe the clearer effects in the starved cells simply reflect the different floor effects of the GFP-LC3 dot counting versus western blotting assays. I wondered whether some comment on this would be helpful in the discussion.

Response to comment 2) We think the referee is exactly correct with this interpretation. Figures 1A and 1B use cells with stable transfection of GFP-LC3 whereas figure 1C examines endogenous LC3. Thus, the model system used for figure 1A/B is likely more sensitive to the subtle changes in autophagy resulting from DENND3 knockdown in the unstarved conditions. In revised figure 1C we have removed the unstarved condition to better emphasize the Bafilomycin A1 results. We now make mention that the influence of DENND3 knockdown on autophagy is subtle in unstarved conditions on page 7 of the main text.
Referee #2

Comment 1) Xu and colleagues describe a regulation of autophagy through Rab12, activated after starvation by DENND3 which they show is a substrate of overexpressed ULKs. They identified the phosphorylation site on DENND3 as binding site for 14-3-3- proteins by mass spec in DENND3 immunoprecipitations. Starvation increases the phosphorylation of DENND3, and activation of Rab12. Finally they show recombinant or overexpressed Rab12 binds LC3.

Previous reports in the literature regarding Rab12, and DENND3, as well as a report that DENND3 binds LC3 all support the authors theory that activated Rab12 may regulate autophagy. However, it is not clear how Rab12 functions, and the data is on the whole preliminary and requires additional experiments both for validation of the existing data and to uncover the mechanism of action of activated Rab12.

Response to comment 1) We agree with the referee that more work will be required to fully understand the role of Rab12 in autophagy. The main focus of the current study is on how ULK regulates DENND3 and thus Rab12 during this process, and we discovered a novel mechanism underlying the role of ULK in autophagy. An important follow-up project is to screen for Rab12 effector proteins, perhaps molecular motors, which will help further elucidate the role of Rab12 in the autophagy process.

We also agree with the referee that the existing data requires additional validation, and we have attempted to deal with all of the referee’s comments as discussed below.

Comment 2) Major points: 1. Figure 1, the formation of GFP-LC3 puncta is clearly influenced by loss of DENND3. On the other hand the endogenous LC3 lipidation and p62 are not conclusive. LC3-lipidation is typically expressed as LC3-II over a housekeeping protein. From the western blots shown LC3-II levels do not look substantially changed and the difference may be due to changes in LC3-I levels. In addition, while p62 degradation does appear to be inhibited by loss of DENND3 in starvation, it is surprising that in the unstarved DENND3 siRNA sample that p62 has not accumulated.

Response to comment 2) As discussed in the response to Referee #1, comment 1, we have improved the quality of the blots in figure 1C and we quantified the blots by normalizing LC3-II levels to GAPDH as suggested. We agree with the referee that the influence of DENND3 knockdown on autophagy markers in the unstarved conditions is very subtle. In the original submission we reported a change in autophagy in unstarved conditions when counting GFP-LC3 puncta but not when examining p62 levels or LC3-II accumulation. Referee #1 suggested that this may be due to different floor effects between the two systems and we agree. Figures 1A and 1B use cells with stable transfection of GFP-LC3 whereas figure 1C examines endogenous LC3 and p62. Thus, the model system used for figure 1A/B is likely more sensitive to the subtle changes in autophagy resulting from DENND3 knockdown in the unstarved conditions. In revised figure 1C we have removed the unstarved condition to better emphasize the Bafilomycin A1 results as discussed in response to comment 1 of referee #1. We now make mention that the influence of DENND3 knockdown on autophagy is subtle in unstarved conditions on page 7 of the main text.

Comment 3) Figure S1 uses chloroquine to inhibit lysosome. See recent paper (Florey et al., 2014 Autophagy) as this is not recommended now as CQ has been shown to induce LC3-lipidation.
Response to comment 3) We thank the referee for noting this important fact. As suggested, we redid the experiments originally reported in figure S1 with Bafilomycin A1. We have now integrated the data from the Bafilomycin A1 experiment into figure 1C and deleted original figure S1.

Comment 4) 2. In Figure 1C, 2C, D, S3A,B,C and 2F the authors show phosphor analysis with anti-pS554. From the CIP data in Fig. S2B this antibody is clearly not phosphor-specific and is not useful. All these panels require normalization to the total DENND3 protein as the protein levels themselves might be altered. In addition the changes in pS554 are minor in Fig 2D considering the interaction with the non-phopho protein.

Response to comment 4) The referee states that the anti-pS554 antibody is not phosphor-specific. While we agree that there is a non-phosphor pool of antibody in the anti-pS554 sample, there is clearly a dominant pool of antibody that is specific for phosphorylated S554. This can be seen by the large decrease in antibody signal with the DENND3 S554A mutation and the CIP treatment (figures S1B and S1C). Unfortunately, despite repeated attempts, we have been unable to generate a pan-DENND3 antibody to normalize protein levels. While it seems unlikely that the levels of DENND3 protein would change in the short serum-starvation treatments used (10-120 min), we have nevertheless performed qPCR, revealing that DENND3 mRNA levels do not change among the various treatments (revised figures 2E, 2K and S2D).

Comment 5) 3. The authors report the ULK has been shown to bind their kinase substrates. The binding they refer to is the interaction of the c-terminal region of ULK 1 with Atg13. This binding has not been shown to regulate the phosphorylation of Atg13. to demonstrate direct binding in Fig S3D, E the authors should show using endogenous proteins the interaction of ULK1 and DENND3, and map the binding sites on both proteins. This could be used to look at mutants of DENND3 that cant bind ULK.

Response to comment 5) Unfortunately, despite repeated attempts we have been unable to generate an antibody that is effective for immunoprecipitation of endogenous DENND3. Moreover, we also do not have access to an antibody for immunoprecipitation of endogenous ULK1. Thus, at this point in time we are unable to test the ULK1/DENND3 interaction at the endogenous level. We have added a comment to this effect in the main text. As suggested by the referee, we have performed some new mapping experiments. Interestingly, we found that epitope-tagged ULK1 binds to the WD40 domain of DENND3 expressed as a GST fusion protein (revised figure S2G). As supported by the Senior Editor, we have not performed further mapping for the DENND3 binding site on ULK.

Comment 6) 4. The rescue experiment shows that each phosphosite alone is required for rescue. This is interesting and has implications for activation, and 14-3--3 binding. This would mean that the phosphor-mimic protein would have to be a S554D,S572D (or E mutants) to rescue like wt.

Response to comment 6) This is an interesting point. Thus, we redid the rescue experiments with a construct in which we converted S to E for both phosphorylation sites of DENND3. This construct does indeed rescue as shown in revised figure 3B.

Comment 7) 4. The authors show in Fig. 3 RILP1 binds activated Rab12. In Fig. 4 A ands B in the presence of GTPgammaS would they predict RIPL1 would be bound? and could this compete for LC3 interaction?
Response to comment 7) RILPL1 is an effector for Rab12, which means it binds selectively to Rab12 in the GTP-bound form. In contrast, as demonstrated in figure 4A, LC3 does not display a nucleotide preference in its interaction with Rab12. Therefore, it seems unlikely that LC3 and RILPL1 would compete for Rab12 interaction. In this manuscript the Rab12 effector RILPL1 is simply used as a tool to assess the levels of active Rab12. We would like to test whether RILPL1 functions in autophagy in coordination with Rab12, but as supported by the Senior Editor, these studies go beyond the scope of the current manuscript.

Comment 8) 5. Where do Rab12 and LC3 interact? is it via a LIR motif in LC3? is Rab12 a cargo degraded in autophagosomes?

Response to comment 8) Considering the relatively weak binding between Rab12 and LC3 (figure 4A) and the fact that there are at least three potential LIR motifs in Rab12, which may function cooperatively for Rab12 interaction, mapping the interaction site on Rab12 will be difficult, and as stated by the Senior Editor, not a prerequisite for this manuscript. According to a recent study (figure 1G of Matsui and Fukuda, EMBO Rep., e2013), Rab12 levels do not change upon starvation, suggesting that Rab12 is not a cargo degraded in autophagy.

Comment 9) 6. Fg. 4 panel D the LC3 would presumably be on membranes if co-localizing with Rab12. An additional informative measure would also include the % of LC3 which was Rab12 negative- this should change in starvation.

We did the quantification as suggested and the data is as follows:

![Graph showing % of LC3 which is Rab12 negative](image)

However, after applying an unpaired t-test for statistical analysis, we found that the difference between unstarved and starved cells is not statistically significant. The experiments were repeated 3 times, n = 61 cells. Bars represent mean ± SEM.

Comment 10) 7. Fig. 4C. There seems to be a large amount of LC3 in the cells overexpressing Rab12. This might indicate that Rab12 actually inhibits maturation of LC3-positive autophagosomes. Flux analysis with BafA should be done in these cells (using conditions which give higher transfection efficiency).

Response to comment 10) According to the report of Matsui and Fukuda, EMBO Rep., 2013, manipulation of Rab12 levels does not change autophagy flux.

Comment 11) Minor 1. A GST control is required in Fig. S4B.

Response to comment 11) We added a GST control as suggested. This is now presented in revised figure S3B.

Comment 12) 2. Are the plasmids used in Fig. 3B siRNA-resistant? if so please supply information.
Response to comment 12) Yes, the plasmids are siRNA-resistant. We apologize for not including this information in the original submission; we have added the relevant information to the supplemental Materials and methods section of the revised manuscript.

Referee #3

Comment 1) This manuscript is very clearly written, and presents high quality data supporting an intriguing model linking starvation to autophagosome formation and autophagy, using beautiful and convincing biochemistry and cell biology approaches. I don't think any additional experiments are necessary for this manuscript to be impactful, interesting and compelling; however, if the authors have in hand some of the results I ask about below, or could add in some text to address some of these questions, it would strengthen this manuscript if they were included.

1. Have starvation times longer than 120 min been applied? I wonder if this signaling pathway is transiently activated, vs. generating a sustained signal that is not down regulated until the stimulus is removed.

Response to comment 1) First, we would like to thank the referee for the very positive statements regarding our study and manuscript. We were also curious as to whether the phosphorylation of DENND3 was transient or could be sustained beyond the 120 min time point we had previously examined (figure 2C). Thus, we looked at 6 h of serum starvation and continued to see elevation of S554 phosphorylation (see reviewer figure below). We made mention of this data on page 9 of the revised manuscript and are not apposed to incorporating this figure into the manuscript.

Comment 2) ULK is described as homologous to the yeast kinase Atg1. Are there other known parallels of the signaling pathway uncovered here with yeast proteins/interactions/pathways?

Response to comment 2) DENND3 only exist in vertebrates. There is no orthologue of DENND3 in yeast. However, as shown in figure 2B, the phosphorylation sites of DENND3 are highly conserved amongst vertebrates, suggesting that the ULK/DENND3 signaling pathway is conserved amongst vertebrates.

Comment 3) Does siRNA of any of the 14-3-3 proteins also affect autophagy? I realize this may be too difficult to do if there is too much redundancy in the 14-3-3 family and their expression in the cells used.
Response to comment 3) We are also curious about the relationship of DENND3 and 14-3-3 proteins in autophagy. However, as mentioned by the referee, 14-3-3 proteins have 7 isoforms and 6 of them bind to DENND3. Due to this redundancy, knocking down only one of the isoforms may not have an influence. In addition, 14-3-3s have more than 200 binding partners. Some of the binding partners are involved in autophagy, including Beclin1, TCS2, PRAS40, Vps34, and Raptor. This complexity will further cloud or ability to explore the relationship between DENND3 and 14-3-3 proteins in autophagy. We have made a comment on this in the main text.

Comment 4) 4. Do the authors have any ideas about how DENND3 and/or Rab12 might lead to activation of the motor proteins that are apparently necessary for mobility that facilitates completion of autophagy? This point in particular should be fleshed out a bit more in the text.

Response to comment 4) In fact, searching for motor proteins that function as effectors for Rab12 will be a main focus of our next study. We have added new discussion regarding the potential relationship between Rab12 and motor proteins in the main text of the revised manuscript.
I have now received the enclosed reports from referees 1 and 2, both of whom are now supportive of the publication of your study in EMBO reports. Referee 2 has some outstanding issues that should be discussed in the text, but no further experimentation will be necessary.

I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- Please address the minor issues raised by referee 2. As you will see below, I am proposing an alternative title that does not mention ULK. However, I would be open to including ULK in the title if you prefer, as I think your work supports this (with the caveats raised by referee 2).

- Figure 2 is larger than 1 page. I must therefore ask you to reassemble it so that it occupies one page maximum. Please note that we can accommodate up to five figures in the main text, which means that you could also split it into two figures and/or move some of the supplement into the main article.

Once all remaining corrections have been attended to, you will receive an official decision letter accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

REFEREE REPORTS:

Referee #1:

I am satisfied with the revisions that have been made. The authors have not been able to make a pan-DENND3 antibody. They have used mRNA levels as a surrogate for total protein levels in their experiments aiming to measure phosphorylation. Given the lack of a suitable reagent, this is a reasonable compromise. This issue should also be considered in the context of the entire paper, and the fact that it is very unlikely that the changes in phosphorylation are due to changes in total protein. I agree with the authors that the phosphatase experiments suggest that the bulk of the protein detected by the phospho-antibodies is phosphorylated.

Referee #2:

While the authors have provided responses to most of my comments (some were ruled out by the EMBO editor) I am still concerned that the conclusion that ULK1 directly interacts with and phosphorylates DENND3 is overstated. Tagged kinases can immunoprecipitate others kinases (eg ULK1 may co-immunoprecipitate mTORC1) and siRNA depletion of kinases (in particular ones in multi-protein complexes such as ULK1/2) might result in unanticipated activation or inhibition of other kinase pathways.

Specific comments:
1. Regarding the response to comment 5.
"Response to comment 5) Unfortunately, despite repeated attempts we have been unable to generate an antibody that is effective for immunoprecipitation of endogenous DENND3. Moreover, we also do not have access to an antibody for immunoprecipitation of endogenous ULK1. Thus, at this point in time we are unable to test the ULK1/DENND3 interaction at the endogenous level. We have added a comment to this effect in the main text."

I was unable to find the comment about the endogenous interaction in the main text. Apologies if I overlooked it but it was not obvious.

2. The conclusions that the ULK1/2 complex directly phosphorylates DENND3 must be toned
down. A statement concerning the lack of absolute proof regarding ULK1 phosphorylation must be added to the text. I feel strongly that it would also be prudent to remove the ULK from the title.

2nd Revision - authors’ response 30 March 2015

Thank you again for your review of our manuscript. We have uploaded a revised version with additional information as listed below.

We decided to use the original title that mentions ULK. We feel that having ULK in the title will bring additional attention to the manuscript.

We have rearranged figure 2 placing all panels on one page.

The referee stated “I was unable to find the comment about the endogenous interaction in the main text. Apologies if I overlooked it but it was not obvious.” This comment was lacking.

We have now added the comment in the first paragraph of page 10.

The referee stated “While the authors have provided responses to most of my comments (some were ruled out by the EMBO editor) I am still concerned that the conclusion that ULK1 directly interacts with and phosphorylates DENND3 is overstated.

Tagged kinases can immunoprecipitate others kinases (eg ULK1 may coimmunoprecipitate mTORC1) and siRNA depletion of kinases (in particular ones in multi-protein complexes such as ULK1/2) might result in unanticipated activation or inhibition of other kinase pathways.”

“2. The conclusions that the ULK1/2 complex directly phosphorylates DENND3 must be toned down. A statement concerning the lack of absolute proof regarding ULK1 phosphorylation must be added to the text. I feel strongly that it would also be prudent to remove the ULK from the title.”

The sum total of the data strongly suggests that ULK directly phosphorylates DENND3. However, the reviewer is correct in that absolute proof is lacking. We have thus added a line on page 10 indicating this fact.

3rd Editorial Decision 31 March 2015

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. It was a pleasure to work with you on this study.