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YME1L Degradation Reduces Mitochondrial Proteolytic Capacity During Oxidative Stress

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Editor: Barbara Pauly

1st Editorial Decision 02 June 2014

Thank you very much for the submission of your research manuscript to our editorial office. We have now received the full set of reviews on your manuscript.

As the detailed reports are pasted below I will only repeat the main points here. You will see that all reviewers appreciate the interest of your findings and are, in principle, supportive of publication of your study in our journal. However, they are also in agreement that several aspects of your data need improvement before the paper can be published. Most importantly, they all point out that the physiological relevance of the proposed regulation of YME1L degradation needs to be explored further and referee 1 states that stronger evidence is need to exclude the possibility that YME1L degradation is not simply a result of programmed cell death. Referee 2 also feels that it should be tested whether mitochondria/cells can recover from stress-induced loss of YME1L and that the kinetics of YME1L/Oma1 degradation should be investigated in more detail. With regard to the mechanistic insights requested by this reviewer, we would not insist on their addition. However, if you can perform some of the experiments suggested by this referee (for example, investigating the effects of PINK1/Parkin depletion or testing the potential involvement of candidate proteases other than Oma1) we would encourage you to add these data.

Overall, and given the reviewers' constructive comments, I would like to give you the opportunity to

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revise your manuscript, with the understanding that the main concerns of the reviewers should be addressed. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

As a minor point, I noticed that on page 9 it says 'The inability of H2O2 to induce YME1L degradation could be attributed to lower levels of eIF2a phosphorylation ....'. Should this not read '...Tim17A degradation...'?

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. If you feel that this period is insufficient for a successful submission of your revised manuscript I can potentially extend this period slightly. Also, the length of the revised manuscript should not exceed roughly 29,000 characters (including spaces and references). While you may consider displaying peripheral results as supplementary information, the materials and methods required for the understanding of the main experiments may not be displayed in the supplementary section only.

I look forward to seeing a revised form of your manuscript when it is ready. Should you in the meantime have any questions, please do not hesitate to contact me.

REFEREE REPORTS:

Referee #1:

In this manuscript the authors investigate the degradation of the mitochondrial peptidase YME1L, which occurs under conditions of acute stress. Using a variety of different cell lines and stresses, the authors demonstrate the proteolytic instability of YME1L is dependent upon the mitochondrial protease OMA1. The authors perform a variety of dump-on experiments using such oxidative insults at hydrogen peroxide, antimycin, and the mitochondrial membrane uncoupler CCCP. They observe that the stability of YME1L but not its paralogue AFG3L2 exhibits a does-dependent stability by western blot analyses. They block the synthesis of cytosolic translation using cyclohexamide to examine the stability of YME1L and observe that the turnover is increased with time in the presence of hydrogen peroxide. Using MEFs genetically ablated for the protease OMA1, the authors report that the stability of YME1L is maintained in the face of some of the same oxidative stresses. As a control for the induction of OMA1, they follow the degradation of long protein isoforms of OPA1, which is a marker of OMA1 proteolysis. In OMA1 knockout embryonic fibroblasts, the stability of OPA1 is maintained in the face of hydrogen peroxide, as is the stability of YME1L. Interestingly, the authors also show that the stability of YME1L is not dependent upon mitochondrial membrane potential, as the protonophore CCCP, which dissipates membrane potential, does not reduce the stability of YME1L despite activating OMA1 (as evidenced by OPA1 turnover). However, it is only with CCCP treatment combined with deoxy-glucose, which is an analogue of glucose that cannot be hydrolyzed and therefore blocks glycolysis, that the stability of YME1l is decreased.

The question of proteolytic stability of YME1L is of great interest, particularly because this protease has recently been shown to play a role in mitochondrial dynamics and protein quality control. Thus, it is of great value to understand how YME1L is regulated a post-translational level. However, the physiological relevance of YME1L degradation remains largely speculative. Therefore, it seems possible that the stress-induced degradation of YME1L incited by the addition of H2O2 and antimycin, triggers apoptosis and thus the turnover of YME1L is simply a consequence of ongoing apoptosis. Indeed all their data would support such a phenomenon: the turnover of long OPA1 isoforms, coincident with YME1L turnover, is known to incite and occur during apoptosis. This turnover can of course be rescued upon ablation of OMA1 and so it is no surprise that the authors are also able to rescue YME1L lability in the OMA1 KO cells.

Additional support that OMA1 activation alone (in the absence of apoptotic induction) is insufficient for the turnover of YME1L is nicely quantified in Figure 2E. Here, the authors are clearly able to incite the stress-induced activation of OMA1 with CCCP, as evidenced by the turnover of OPA1,

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yet the stability of YME1L is unaffected. It is important to note that CCCP addition does not necessarily trigger apoptosis. Only upon additional glycolytic inhibition, which one could envision triggers programmed cell death, does the stability of YME1L decrease. Hence, the interpretation of these data could be that OMA1 turns over YME1l only during apoptosis.

It is unclear what the actual cellular consequence the degradation of YME1L under these conditions is. The authors show that YME1L loss coincides with increased apoptosis in the presence of As(III). However, it is important to know whether the degradation of YME1L is a specific event occurring during apoptosis or whether it is one of many mitochondrial proteins subjected to proteolysis (by OMA1 or other proteases).

Additional points:
1. YME1L loss has already been reported to cause apoptotic sensitivity, ultrastructural changes of cristae, and mitochondrial fragmentation. Does YME1L loss precede the induction of such phenotypes in the presence of stress? This is not clear from Figure 4 as As(III) concentrations inciting cell death are varied whereas in Figure 1D it is the incubation time in As(III) that is varied. If these phenotypes are attributed to YME1L alone, then OMA1 depletion should be capable of reversing them?
2. It is clear from the authors' data that a protease other than OMA1 contributes to YME1L degredation. Which protease could this be and does it depend on OMA1 for function?
3. In isolated mitochondria the authors demonstrate that exogenously added ATP increases the stability of YME1L over time and they go on to show it is ATP and ADP but not AMP that can impinge upon this degradation event. Does this imply that the binding of nucleotides as substrates stabilizes YME1L folding preventing degradation?
4. Figure 4A is labeled p-eIF2! And tot-eIF2! Are these labels correct? The "!" mark?

Referee #2:

In this study the authors characterize the stress-induced degradation of the mitochondrial inner membrane protease Yme1. This process depends, at least partly, on another inner membrane protease Oma1, and is activated by low ATP levels and a loss in potential. Recent studies have also shown that Oma1 is degraded upon stress, in that case the authors suggested that this downregulation allows the mitochondria to recover through import of new Opa1, for example (EMBO Rep. 2014 May 1;15(5):576-85.; EMBO J. 2014 Mar 18;33(6):578-93). In this case, however, there is no clear answer as to why, or even how Yme1 is degraded. The only substrate examined was Tim17, although not a great deal of insight was offered from those experiments except to confirm that cyclohexamide dependent Tim17 degradation required Yme1. The only functional implication is that the loss of Yme1 increases the susceptibility of stressed cells to die. This is also not a new finding, but the authors use it to explain why Yme1 would be actively degraded.

Overall, the emerging complexity of the protease casacades in the regulation of mitochondrial quality control is of great importance in the field. That a "housekeeping" protease like Yme1 is degraded is new and interesting, however the current manuscript offers little in the way of mechanisms or physiological relevance. For the readership of EMBO Reports, the authors should address the following experimental points:

1. If the authors remove the stressors does Yme1 import resume and mitochondria recover? Or is the loss of Yme1 reflecting a "point of no return"? If Oma1 is required for it's cleavage, then Yme1 loss should precede the loss of Oma1, and since Oma1 cleavage is reversible, so should Yme1 cleavage. On the other hand, if Yme1 activity is required for Opa1 processing following import (J Cell Biol. 2007 Aug 27;178(5):757-64 and J Cell Biol. 2007 Aug 27;178(5):749-55.), how does it recover?
2. I realize that there is no commercially available Oma1 antibody to correlate the loss of Yme1 with the (subsequent?) loss of Oma1. However, the authors could use a tagged form or acquire the antibody from the Langer group. It is important to define the kinetics of these events. Opa1 cleavage is used as a sensor for Oma1 function, but since Oma1 is to be degraded, this is a separate issue from Opa1. For example, in figure 2, the Oma1+/+ cells show loss of AFG3L2, Yme1 and Opa1 cleavage by 2 hours with 100-200uM peroxide. The kinetics could be more informative if either the

dose is reduced (200uM is very high, as is 50uM CCCP...), or the time intervals increased so we can see the order of events, and relate that to Oma1 degradation.
3. Concerning the stress triggers, it is intriguing that 100nM antimycinA led to robust Yme1 loss since this is unlikely to depolarize and may even lead to a hyperfused response at this dose. What do the mitochondria look like in 100nM antiA treatments on galactose (Fig 1F)? This should not have triggered significant Parkin recruitment (see next point) and could be expanded upon to derive mechanism. It's unclear why the bulk of the experiments were done with very high levels of peroxide, which is really quite severe, and the effects on Yme1 don't seem as robust or specific as Fig1F (compare with Fig 1B, for example where AFG3L2 is also lost, and only at highest concentrations).
4. The suggestion is that Oma1 acts along with another unidentified protease, which is certainly logical. However, recent work in both yeast and mammalian systems has hinted towards mechanisms of selective mitophagy (Nat Commun. 2013;4:2789.), or vesicle transport (EMBO J. 2014 Feb 18;33(4):282-95.). These pathways depend on PINK1 and Parkin in mammalian systems. If the authors silence Parkin and PINK1, does Yme1 degradation still occur? Do the authors observe any overlay among the protease cascades and mitophagy? The kinetics of Parkin recruitment (generally 30-90 minutes) would suggest that the protease degradation occurs while mitochondria are undergoing mitophagy (in the peroxide or CCCP treatments at least). In vitro assays suggest the answer is no, but it should be tested formally. Where do the authors think the tipping point is for this protease cascade relative to mitophagy? If Yme1 is cleaved, is this when PINK1 may accumulate, for example? This type of question could be nicely addressed in the discussion to lend context to the non-expert readers.
5. It is unexpected that the megadalton complex shows no sign of disassembly on the way to degradation. It is rather like it just disappeared. Only one time point was examined, 6 hours following 100uM peroxide. Are intermediates caught at earlier time points? If the authors add the protease inhibitors do they observe any accumulation of altered intermediates?
6. The authors should directly address the potential role of LonP, MPPs or the Clp proteases in the turnover of Yme1. They should really all be screened as was done for the cleavage of Opa1 and PINK1 over the last number of years by various authors.
7. There is discussion about a potential accumulation of oxidized proteins after the time when Yme1 is degraded. It is important to test this, which can be done by silencing Oma1 to protect against Yme1 loss, and perform oxyblot experiments to monitor the accumulation of oxidized proteins in cells where Yme1 is lost (control, with stress), or not (Oma1-/-).

Referee #3:

Overall, an interesting paper that addresses an important question regarding mitochondrial proteolysis regulation. The authors report a novel mechanism of regulation of YME1L stability, through oxidative stress combined with nucleotide depletion. They show that OMA1 is a major protease involved in YME1L degradation and their results suggest that this degradation of YME1L is involved in cell death resulting from oxidative stress. This final point, which demonstrates the biological relevance of this study is important and is not well supported by the data. Further experimental support is needed to strengthen this study. Specifically the authors should address the following:

1. The major concern is the lack of solid experimental data demonstrating the biological relevance. The authors should confirm that YME1L degradation is important in cell sensitivity in response to oxidative stress, by showing that preventing the degradation of YME1L can enhance cell survival in response to stress, and this should be demonstrated several ways in more than one cell type. Does over expression of YME1L improve survival after oxidative stress?
2. Blue native gel is poor quality (Fig.2G) - better gel should be provided.
3. The data does not fully support the model shown in Fig.3G indicating that YME1L is destabilized, infact this model is inconsistent with the data in Fig. 2G. Model should be amended appropriately.

Overall, the biochemistry is well done however the biological relevance needs to be solidified and a few figures should be improved.

1st Revision - authors' response 02 September 2014

EDITOR COMMENTS.

Editor General Comments: *"Thank you very much for the submission of your research manuscript to our editorial office. We have now received the full set of reviews on your manuscript.*

As the detailed reports are pasted below I will only repeat the main points here. You will see that all reviewers appreciate the interest of your findings and are, in principle, supportive of publication of your study in our journal. However, they are also in agreement that several aspects of your data need improvement before the paper can be published. Most importantly, they all point out that the physiological relevance of the proposed regulation of YME1L degradation needs to be explored further and referee 1 states that stronger evidence is need to exclude the possibility that YME1L degradation is not simply a result of programmed cell death. Referee 2 also feels that it should be tested whether mitochondria/cells can recover from stress-induced loss of YME1L and that the kinetics of YME1L/Oma1 degradation should be investigated in more detail. With regard to the mechanistic insights requested by this reviewer, we would not insist on their addition. However, if you can perform some of the experiments suggested by this referee (for example, investigating the effects of PINK1/Parkin depletion or testing the potential involvement of candidate proteases other than Oma1) we would encourage you to add these data.

Overall, and given the reviewers' constructive comments, I would like to give you the opportunity to revise your manuscript, with the understanding that the main concerns of the reviewers should be addressed. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript."

Our Response to Editor General Comments: We thank the editor and the reviewers for the careful review of our manuscript and the opportunity to submit a revised version. In the revised manuscript, we include 12 new figures and adapted text to address the reviewer's valuable comments. We describe these changes to the manuscript below in our point-by-point response to the reviewers' comments. We believe that our manuscript has been significantly improved by your and the reviewers' valuable comments and the review process at *EMBO Reports.*

Editor Minor Comment #1: *"As a minor point, I noticed that on page 9 it says 'The inability of H2O2 to induce YME1L degradation could be attributed to lower levels of eIF2a phosphorylation ....'. Should this not read '...Tim17A degradation...'?"*

Our Response to Editor Minor Comment #1. You are correct. We have changed this phrase in the revised manuscript, as below:

Page 10, Paragraph 1, Line 1: *"The inability for H2O2 to induce Tim17A degradation could be attributed to lower levels of eIF2α phosphorylation induced by this treatment, as compared to As(III)."*

REVIEWER #1

Reviewer #1 General Comments. *"In this manuscript the authors investigate the degradation of the mitochondrial peptidase YME1L, which occurs under conditions of acute stress. Using a variety of different cell lines and stresses, the authors demonstrate the proteolytic instability of YME1L is dependent upon the mitochondrial protease OMA1. The authors perform a variety of dump-on experiments using such oxidative insults at hydrogen peroxide, antimycin, and the mitochondrial membrane uncoupler CCCP. They observe that the stability of YME1L but not its*

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paralogue AFG3L2 exhibits a does-dependent stability by western blot analyses. They block the synthesis of cytosolic translation using cyclohexamide to examine the stability of YME1L and observe that the turnover is increased with time in the presence of hydrogen peroxide. Using MEFs genetically ablated for the protease OMA1, the authors report that the stability of YME1L is maintained in the face of some of the same oxidative stresses. As a control for the induction of OMA1, they follow the degradation of long protein isoforms of OPA1, which is a marker of OMA1 proteolysis. In OMA1 knockout embryonic fibroblasts, the stability of OPA1 is maintained in the face of hydrogen peroxide, as is the stability of YME1L. Interestingly, the authors also show that the stability of YME1L is not dependent upon mitochondrial membrane potential, as the protonophore CCCP, which dissipates membrane potential, does not reduce the stability of YME1L despite activating OMA1 (as evidenced by OPA1 turnover). However, it is only with CCCP treatment combined with deoxy-glucose, which is an analogue of glucose that cannot be hydrolyzed and therefore blocks glycolysis, that the stability of YME1l is decreased.

The question of proteolytic stability of YME1L is of great interest, particularly because this protease has recently been shown to play a role in mitochondrial dynamics and protein quality control. Thus, it is of great value to understand how YME1L is regulated a post-translational level."

Our Response to Reviewer #1 General Comments. We thank the reviewer for the careful reading of our manuscript. We address all of Reviewer #1's valuable comments in our revised manuscript, as described below.

Reviewer #1 Comment #1. *"The question of proteolytic stability of YME1L is of great interest, particularly because this protease has recently been shown to play a role in mitochondrial dynamics and protein quality control. Thus, it is of great value to understand how YME1L is regulated a post-translational level. However, the physiological relevance of YME1L degradation remains largely speculative. Therefore, it seems possible that the stress-induced degradation of YME1L incited by the addition of H2O2 and antimycin, triggers apoptosis and thus the turnover of YME1L is simply a consequence of ongoing apoptosis. Indeed all their data would support such a phenomenon: the turnover of long OPA1 isoforms, coincident with YME1L turnover, is known to incite and occur during apoptosis. This turnover can of course be rescued upon ablation of OMA1 and so it is no surprise that the authors are also able to rescue YME1L lability in the OMA1 KO cells.*

Additional support that OMA1 activation alone (in the absence of apoptotic induction) is insufficient for the turnover of YME1L is nicely quantified in Figure 2E. Here, the authors are clearly able to incite the stressinduced activation of OMA1 with CCCP, as evidenced by the turnover of OPA1, yet the stability of YME1L is unaffected. It is important to note that CCCP addition does not necessarily trigger apoptosis. Only upon additional glycolytic inhibition, which one could envision triggers programmed cell death, does the stability of YME1L decrease. Hence, the interpretation of these data could be that OMA1 turns over YME1l only during apoptosis."

Our Response to Reviewer #1 Comment #1. The reviewer is specifically asking whether YME1L degradation is a '*consequence of ongoing apoptosis*'. We have addressed this concern in the revised manuscript by monitoring the activation of apoptotic signaling pathways under conditions that induce YME1L degradation. In these experiments, we find that the activation of apoptotic signaling (shown by caspase-3 and PARP cleavage) does not correlate with YME1L degradation. The addition of H2O2 (200 μM) to N2a cells does not induce apoptosis, but results in a robust rapid reduction in YME1L protein levels (see revised **Figure 4A**). While previous results have shown H2O2 induces apoptosis in certain cells (e.g., 200 μM H2O2 in HEK293T cells as shown in Stiburek et al (2012) MBoC), our results shown in **Figure 4A** are consistent with other published manuscripts showing that H2O2 induces non-apoptotic cell death in other cell lines (Vaseva et al (2012) Cell and Vanden Berghe et al (2009) Cell Death & Diff). The addition of antimycin A to cells cultured in galactose supplemented media – a condition that induces selective YME1L degradation – also does not lead to increased caspase-3 activation or PARP cleavage (see revised **Figure S3B)**. Alternatively, cells treated with As(III) induce both apoptosis and YME1L degradation (see revised **Figure S4A**). Furthermore, we show that staurosporine – a molecule that induces robust apoptosis – does not significantly

impact YME1L stability (**Figure 4A**). Collectively, these results show that YME1L degradation is not a consequence of ongoing apoptosis. We address this important point in the revised manuscript as below:

Page 9, Paragraph 2, Line 1: *"Stress-induced YME1L degradation could represent an early step in apoptotic signaling. To test this prediction, we monitored apoptotic signaling by caspase-3 activation and PARP cleavage in cells treated with stresses that induce YME1L degradation. Despite inducing robust YME1L degradation, we observe no caspase-3 activation or PARP cleavage in N2a cells treated with H2O2 (**Figure 4A**) or HEK293T cells cultured in galactosesupplemented media and treated with antimycin A (**Figure S3B**). Conversely, staurosporine robustly activates apoptosis without significantly affecting YME1L stability (**Figure 4A**). Alternatively, YME1L degradation, caspase-3 activation and PARP cleavage are all observed in As(III)-treated SHSY5Y cells (**Figure S4A**). These results indicate that stress-induced YME1L degradation does not correspond with the initiation of apoptosis."*

Additionally, we include new data in the revised manuscript showing that YME1L protein levels recover over a 24 h period following a 6 h treatment with H2O2 (see revised **Figure 4B**). This further supports our data showing that YME1L degradation does not result from ongoing apoptosis nor does it represent a terminal event in cellular survival. We discuss these new results in the revised manuscript as below:

Page 9, Paragraph 2, Line 9: *"Furthermore, YME1L protein levels recover over a 24-hour period following a H2O2 insult, mirroring the recovery of full length OPA1 (**Figure 4B**) [29]. This suggests that YME1L degradation does not represent a 'point of no return' for cell death."*

Reviewer #1 Comment #2. *"It is unclear what the actual cellular consequence the degradation of YME1L under these conditions is. The authors show that YME1L loss coincides with increased apoptosis in the presence of As(III). However, it is important to know whether the degradation of YME1L is a specific event occurring during apoptosis or whether it is one of many mitochondrial proteins subjected to proteolysis (by OMA1 or other proteases)."*

Our Response to Review #1 Comment #2. As discussed above, we show that YME1L degradation is not a consequence of ongoing apoptosis (see **Our Response to Reviewer #1 Comment #1**). This indicates that YME1L degradation is not an early event in apoptotic signaling. Instead, we evaluate the impact of stressinduced YME1L degradation on the capacity for cells to regulate inner membrane proteostasis, specifically focusing on the model YME1L substrate Tim17A. We show that stress-regulated Tim17A degradation is inhibited by H2O2 (see revised **Figure 4C** and **D**). Additionally, we provide new data in the revised manuscript showing that *TIM17A* depletion in HEK293T cells, which mimics stress-regulated Tim17A degradation, increases the viability of cells treated with H2O2 (see revised **Figure 4G**). Collectively, these results indicate stress-induced YME1L degradation decreases YME1L proteolytic activity and prevents protective stressregulated remodeling of mitochondrial proteostasis. This decreased capacity to regulate mitochondrial proteostasis sensitizes cells to pathologic insults, as reflected by the increased sensitivity of *YME1L*-depleted cells to stresses such as staurosporine and arsenite (As(III)) (see revised **Figure 4E,F** and **S4D**). Overall, the results shown in **Figure 4** and **S4** of the revised manuscript support a model whereby the stress-induced YME1L degradation sensitizes cells to oxidative insults by decreasing the capacity to regulate mitochondrial inner membrane proteostasis through functions such as Tim17A degradation. We discuss these results in the revised manuscript, as below:

Page 10, Paragraph 2, Line 5: *"This suggests that the loss of YME1L-dependent regulation of mitochondrial proteostasis (such as Tim17A degradation) sensitizes cells to oxidative insult. Consistent with this prediction, depletion of TIM17A in HEK293T cells – mimicking stressregulated Tim17A degradation – increases cellular viability in response to H2O2, suggesting that inhibition of YME1L-dependent Tim17A degradation reduces cellular capacity to regulate mitochondria in response to oxidative insult (**Figure 4G**)."*

The reviewer also is interested in the role for OMA1 in degrading other mitochondrial proteins during stress.

We show that OMA1 is involved in the degradation of the m-AAA protease AFGL32 in response to H2O2 treatment. Alternatively, other mitochondrial inner membrane proteins, such a Tim23 and Tim17A, are not affected by H2O2-dependent OMA1 activation (see revised **Figure S1A** and **4C**). Furthermore, we show that other stresses such as antimycin A treatment of cells cultured in galactose-supplemented media lead to selective degradation of YME1L, without impacting AFG3L2 stability (see **Figure 1F**). Furthermore, we show that other stresses (e.g., CCCP) activate OMA1, but do not lead to robust YME1L degradation (see **Figure 3A**). Collectively, these results suggest that OMA1 is likely involved in degrading other mitochondrial inner membrane proteins during stress, although the complement of proteins subject to this degradation is varied based on the stress. As suggested by the reviewer, we are continuing to explore the impact of OMA1 on the stability of the mitochondrial proteome using other approaches such as quantitative proteomics in work outside the scope of this initial report.

Reviewer #1 Comment #3. *"YME1L loss has already been reported to cause apoptotic sensitivity, ultrastructural changes of cristae, and mitochondrial fragmentation. Does YME1L loss precede the induction of such phenotypes in the presence of stress? This is not clear from Figure 4 as As(III) concentrations inciting cell death are varied whereas in Figure 1D it is the incubation time in As(III) that is varied. If these phenotypes are attributed to YME1L alone, then OMA1 depletion should be capable of reversing them?"*

Our Response to Reviewer #1 Comment #3. As discussed in **Our Response to Reviewer #1 Comment #1**, we now show that YME1L degradation is not attributed to ongoing apoptotic signaling. In order to demonstrate the impact of reduced YME1L on mitochondrial proteostasis, we focused on defining the impact of stressinduced YME1L degradation on a protective YME1L-dependent function, specifically Tim17A degradation. We show that H2O2 treatment attenuates YME1L-dependent Tim17A degradation in cells (**Figure 4C,D**), but not in isolated mitochondria (**Figure S4C**), indicating that stress-induced YME1L degradation inhibits protective YME1L proteolytic activities such as Tim17A degradation. Additionally, we provide new evidence that RNAidepletion of *TIM17A* in HEK293T cells – mimicking the stress-regulated Tim17A degradation inhibited by H2O2 treatment – increases cellular viability when challenged with H2O2 (**Figure 4G**). Collectively, these results are consistent with a model whereby YME1L degradation attenuates protective YME1L-dependent regulation of inner membrane proteostasis. We discuss these new results in the revised manuscript, as below:

Page 9, Paragraph 3, Line 7: *"Despite inducing eIF2α phosphorylation [31], Tim17A is not degraded in H2O2-treated SHSY5Y cells (**Figure 4C**)."*

Page 10, Paragraph 1, Line 5: *"Pretreatment with H2O2 attenuates CHX-dependent Tim17A degradation, consistent with H2O2-induced YME1L degradation inhibiting Tim17A regulation (**Figure 4D**)."*

Page 10, Paragraph 2, Line 5: *"This suggests that the loss of YME1L-dependent regulation of mitochondrial proteostasis (such as Tim17A degradation) sensitizes cells to oxidative insult. Consistent with this prediction, depletion of TIM17A in HEK293T cells – mimicking stressregulated Tim17A degradation – increases cellular viability in response to H2O2, suggesting that inhibition of YME1L-dependent Tim17A degradation reduces cellular capacity to regulate mitochondria in response to oxidative insult (**Figure 4G**)."*

With regards to mitochondrial fragmentation, we show in the revised manuscript that YME1L degradation can be separated from OMA1-dependent OPA1 processing required for mitochondrial fragmentation. Cells treated with CCCP results in rapid OPA1 processing without significant effects on YME1L stability (see revised **Figure 3C**). This stress is well established to induce mitochondrial fragmentation. Similarly, incubating isolated mitochondria in the presence of ATP also results in OPA1 processing without inducing YME1L degradation (see revised **Figure 2F**). Since OPA1 processing correlates with mitochondrial fragmentation, these results indicate that OPA1 processing and YME1L degradation are distinct events induced during stress.
Interestingly, we find that conditions that induce efficient OPA1 processing in the absence of

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YME1L degradation (e.g., CCCP) lead to the rapid degradation of OMA1 (see revised **Figure 3C**). This suggests that the inability for these insults to induce robust YME1L degradation could in part be attributed to reduced levels of active OMA1. Furthermore, this indicates that OPA1 processing-dependent fragmentation and YME1L degradation occur on distinct timescales wherein OPA1 processing occurs rapidly following stress and YME1L degradation occurs at later timepoints. Consistent with this model, we see significant OPA1 processing at 3 h in cells co-treated with 2-DG and CCCP where only a modest amount of YME1L degradation is observed (see revised **Figure 3C**). Intriguingly, our results showing differential stability of OMA1 and YME1L to different stressors could reflect distinct cellular capacities to adapt mitochondrial inner membrane proteolytic activity to specific types of cellular insults. We address this point in the revised manuscript, as below:

Page 8 Paragraph 2, Line 13: *"These results show that OMA1 and YME1L degradation are differentially sensitive to cellular stresses, potentially serving as a mechanism to adapt mitochondrial inner membrane proteolytic activity in response to specific pathologic insults."*

Finally, it is currently difficult to define the specific impact of stress-induced YME1L degradation on any aspect of mitochondrial proteostasis or morphology. The experiment suggested by the reviewer using OMA1 depletion is challenged by the fact that OMA1 knockout does not completely inhibit YME1L degradation and the types of stresses used to induce YME1L degradation such as As(III) disrupt many aspects of cellular physiology apart from just mitochondrial function. In order to address the issues brought up by the reviewer, new methodologies must be developed to sensitively control YME1L stability and/or activity in situ. We are currently developing new genetic and chemical biologic approaches to further define the specific impact of stress-induced YME1L degradation on mitochondrial proteostasis, identified in this initial manuscript.

Reviewer #1 Comment #4. ***"****It is clear from the authors' data that a protease other than OMA1 contributes to YME1L . Which protease could this be and does it depend on OMA1 for function?"*

Our Response to Reviewer #1 Comment #4. The results shown in revised **Figure 2D-F** and **Figure S2E** demonstrate that the loss of OMA1 in MEF cells significantly attenuates, but does not completely inhibit H2O2-induced YME1L degradation. This indicates that another protease is likely involved in this process. Critically, the additional proteolytic activity required for YME1L degradation is independent of OMA1 activity, as we observe this residual, OMA1-independent degradation in OMA1-/- cells, directly addressing the reviewer's comments above.
In the revised submission, we have performed an additional experiment to attempt to identify other mitochondrial proteases that contribute to OMA1-independent YME1L degradation. We show that treating isolated mitochondria with inhibitors of serine, cysteine, or aspartic acid proteases does not attenuate ATPindependent YME1L degradation (see revised **Figure S2D**). This experiment is analogous to that used to define the mechanism of stress-induced OMA1 degradation (Baker *et al.* **2014**. *EMBO J*). Alternatively, the metalloprotease inhibitors *o*-phenanthroline and EDTA both inhibit ATP-independent YME1L degradation. These results, in combination with our results showing that YME1L degradation is an ATP-independent process, suggest that YME1L degradation does not involve the activity of central ATP-dependent quality control proteases such as LON, CLPP, or AFG3L2. We discuss these new results in the revised manuscript, as below:

Page 6, Paragraph 2, Line 1: *"YME1L degradation in isolated mitochondria incubated in the absence of ATP is also inhibited by the zinc chelator o-phenanthroline (o-phe) and the divalent cation chelator EDTA (**Figure 2C**). Inhibitors of serine, cysteine, and aspartic acid proteases did not inhibit YME1L degradation in isolated mitochondria (**Figure S2D**). This suggests that YME1L degradation involves the activity of an ATP-independent zinc metalloprotease. Specifically, a metalloprotease with an active site oriented towards the intermembrane space (IMS), as EDTA cannot cross the inner mitochondrial membrane and only inhibits metalloproteases with IMS-oriented active sites [21]."*

Our result shown in **Figure 2F** indicate that YME1L degradation in isolated mitochondria can

be completely inhibited by the zinc metalloprotease inhibitors o-phenanthroline. This suggests that the other proteolytic activity involved in YME1L degradation is either a zinc metalloprotease or dependent on the function of such a protease. One possibility is that YME1L is able to mediate its own self-cleavage independent of ATP.
Unfortunately, to date, we have been unable to overexpress sufficient amounts of a tagged catalytically inactive YME1L mutant to evaluate the potential involvement of YME1L self-cleavage in this process. We are continuing to explore this (and other) mechanistic aspects of YME1L degradation using other genetic and chemical biologic approaches to influence YME1L activity and recombinant YME1L, which we will report in follow-up manuscripts to this initial report.

Reviewer #1 Comment #4. *"In isolated mitochondria the authors demonstrate that exogenously added ATP increases the stability of YME1L over time and they go on to show it is ATP and ADP but not AMP that can impinge upon this degradation event. Does this imply that the binding of nucleotides as substrates stabilizes YME1L folding preventing degradation?"*

Our Response to Reviewer #1 Comment #4. Yes. Our in vitro results showing that the addition of exogenous ATP, AMP or the non-hydrolyzable ATP analog AMP-PNP suggests that YME1L binding to nucleotide stabilizes YME1L against proteolytic degradation. We address this point in the revised manuscript, as below:

Page 6, Paragraph 1, Line 6: *"These results suggest that binding of nucleotide stabilizes YME1L against degradation independent of ATP hydrolysis."*

Reviewer #1 Comment #5. *"Figure 4A is labeled p-eIF2! And tot-eIF2! Are these labels correct? The "!" mark?"*

Our Response to Reviewer #1 Comment #5. No, the legend should read eIF2α. We apologize for the aberrant "!" introduced by a file conversion error in **Figure 4A** our original submission. We have corrected this error in the revised manuscript (see revised **Figure 4C**).

REVIEWER #2.

Reviewer #2 General Comments: *"In this study the authors characterize the stress-induced degradation of the mitochondrial inner membrane protease Yme1. This process depends, at least partly, on another inner membrane protease Oma1, and is activated by low ATP levels and a loss in potential. Recent studies have also shown that Oma1 is degraded upon stress, in that case the authors suggested that this downregulation allows the mitochondria to recover through import of new Opa1, for example (EMBO Rep. 2014 May 1;15(5):576-85.; EMBO J. 2014 Mar 18;33(6):578-93). In this case, however, there is no clear answer as to why, or even how Yme1 is degraded. The only substrate examined was Tim17, although not a great deal of insight was offered from those experiments except to confirm that cyclohexamide dependent Tim17 degradation required Yme1. The only functional implication is that the loss of Yme1 increases the susceptibility of stressed cells to die. This is also not a new finding, but the authors use it to explain why Yme1 would be actively degraded.*

Overall, the emerging complexity of the protease casacades in the regulation of mitochondrial quality control is of great importance in the field. That a "housekeeping" protease like Yme1 is degraded is new and interesting, however the current manuscript offers little in the way of mechanisms or physiological relevance. For the readership of EMBO Reports, the authors should address the following experimental points:"

Our Response to Reviewer #2 General Comments: We thank the reviewer for the careful reading and critique of our manuscript. We address the reviewer's valuable comments in our revised manuscript, as described below in our point-by-point response. Notably, we provide additional information directly demonstrating the relationship between OMA1 degradation and YME1L degradation during conditions of stress, as discussed in **Our Response to**

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Reviewer #2 Comment #2. Furthermore, we provide new data further demonstrating that YME1L degradation decreases cellular capacity to regulate mitochondrial inner membrane proteostasis, as described in **Our Response to Reviewer #2 Comment #7**. Finally, we have included additional text into the revised manuscript that discusses the interrelationship between YME1L degradation and mitochondrial quality control that begins to highlight the importance of regulating the '*protease cascades*' brought up by the reviewer. Examples of these changes are included below:

Page 8 Paragraph 2, Line 13: *"These results show that OMA1 and YME1L degradation are differentially sensitive to cellular stresses, potentially serving as a mechanism to adapt mitochondrial inner membrane proteolytic activity in response to specific pathologic insults."*

Page 11, Paragraph 1, Line 3: *"Reducing YME1L activity compromises the stress-dependent degradation of YME1L substrates such as Tim17A, impairing mitochondrial capacity to regulate inner mitochondrial membrane proteostasis and function during stress (**Figure 4G**). Interestingly, it was recently shown that YME1L activity is needed for the de novo cleavage of OPA1 to mediate fusion events [10]. Therefore, YME1L degradation may also provide a mechanism to sequester terminally damaged mitochondrial away from the healthy pool thereby restricting them to clearance via mitophagy. The deficiencies in mitochondrial proteostasis and quality control caused by loss of YME1L increase cellular sensitivity to subsequent oxidative stress, as indicated by the increased stress-sensitivity of YME1L-depleted cells."*

Reviewer #2 Comment #1. *"If the authors remove the stressors does Yme1 import resume and mitochondria recover? Or is the loss of Yme1 reflecting a "point of no return"? If Oma1 is required for it's cleavage, then Yme1 loss should precede the loss of Oma1, and since Oma1 cleavage is reversible, so should Yme1 cleavage. On the other hand, if Yme1 activity is required for Opa1 processing following import (J Cell Biol. 2007 Aug 27;178(5):757-64 and J Cell Biol. 2007 Aug 27;178(5):749-55.), how does it recover?"*

Our Response to Reviewer #2 Comment #1. In the revised manuscript, we now show that YME1L protein levels recover following an acute 6 h oxidative insult (H2O2) over a 24 h timecourse (see new **Figure 4B** of the revised manuscript). This recovery is similar to that observed for the recovery of full length OPA1. We address this new Figure in the revised manuscript, as below:

Page 9, Paragraph 2, Line 9: *"Furthermore, YME1L protein levels recover over a 24-hour period following a H2O2 insult, mirroring the recovery of full length OPA1 (**Figure 4B**) [29]. This suggests that YME1L degradation does not represent a 'point of no return' for cell death."*

With respect to the relationship between OMA1 cleavage and YME1L degradation, we address this in detail below in **Our Response to Reviewer #2 Comment #2.**

Reviewer #2 Comment #2. *"I realize that there is no commercially available Oma1 antibody to correlate the loss of Yme1 with the (subsequent?) loss of Oma1. However, the authors could use a tagged form or acquire the antibody from the Langer group. It is important to define the kinetics of these events. Opa1 cleavage is used as a sensor for Oma1 function, but since Oma1 is to be degraded, this is a separate issue from Opa1. For example, in figure 2, the Oma1+/+ cells show loss of AFG3L2, Yme1 and Opa1 cleavage by 2 hours with 100-200uM peroxide. The kinetics could be more informative if either the dose is reduced (200uM is very high, as is 50uM CCCP...), or the time intervals increased so we can see the order of events, and relate that to Oma1 degradation."*

Our Response to Reviewer #2 Comment #2. We have obtained a commercially available OMA1 antibody, recently reported to recognize endogenous human OMA1 (Zhang and Song. **2014**. *EMBO Rep*). We used this antibody to monitor the relative timecourses for the loss of OMA1 and YME1L in response to oxidative stress. As reported previously, we found that CCCP rapidly induces OMA1 degradation with a t50 << 3 h (see revised **Figure 3C**), consistent with the lack of YME1L degradation induced following CCCP treatment. Interestingly, co-administration of CCCP and 2-deoxyglucose significantly slows OMA1 degradation, resulting in the accumulation of a proteolytic fragment previously shown to be an active OMA1

protease (see revised **Figure 3C**). These results are consistent with the robust YME1L degradation observed under these conditions and indicate that OMA1 degradation requires cellular ATP – a result supported by our results from isolated mitochondria showing the degradation of OMA1 requires the addition of exogenous ATP, where YME1L is not degraded (see revised **Figure S3A**). Furthermore, we show that other stresses that induce YME1L degradation also do not significantly influence OMA1 degradation. H2O2 does not induce rapid OMA1 degradation (see revised **Figure 3D**). Similarly, treatment of cells grown in galactose-supplemented media with antimycin A, which induces selective YME1L degradation, also did not induce significant OMA1 degradation (see revised **Figure S3B**), although OMA1 did undergo processing to the cleaved active OMA1 protease. These results clearly demonstrate that conditions that induce robust YME1L degradation (i.e., ATP depletion and membrane depolarization) do not lead to the degradation of OMA1. We address these new results in the revised manuscript, as below:

Page 8, Paragraph 2, Line 1: *"OMA1 undergoes rapid degradation in response to stresses that depolarize the mitochondrial membrane such as CCCP [22, 28]. Thus, we evaluated the relationship between OMA1 and YME1L degradation induced by CCCP in the presence or absence of 2-DG. As observed previously, CCCP induces a reduction in total OMA1 protein levels (**Figure 3C**) [22, 28]. Alternatively, the co-addition of 2-DG with CCCP slows OMA1 degradation and results in the accumulation of a proteolytically processed OMA1 isoform previously shown to retain protease activity (arrows in **Figure 3C**) [28]. The addition of 2-DG alone does not influence OMA1 stability. These results suggest that CCCP-dependent OMA1 degradation is sensitive to cellular ATP levels. Consistent with this prediction, efficient OMA1 degradation in isolated mitochondria requires the addition of exogenous ATP (**Figure S3A**). Other stresses that induce YME1L degradation such as H2O2 and antimycin A in galactosecultured cells also do not induce OMA1 degradation (**Figure 3D** & **S3B**), although antimycin A promotes OMA1 cleavage into the active proteolytic fragment. The activation of OMA1 in antimycin-treated cells cultured in galactose is consistent with the depolarization of the mitochondrial membrane in these cells, as shown by reduction in TMRE fluorescence (**Figure S3C**). These results show that OMA1 and YME1L degradation are differentially sensitive to cellular stresses, potentially serving as a mechanism to adapt mitochondrial inner membrane proteolytic activity in response to specific pathologic insults."*

Reviewer #2 Comment #3. *"Concerning the stress triggers, it is intriguing that 100nM antimycinA led to robust Yme1 loss since this is unlikely to depolarize and may even lead to a hyperfused response at this dose. What do the mitochondria look like in 100nM antiA treatments on galactose (Fig 1F)? This should not have triggered significant Parkin recruitment (see next point) and could be expanded upon to derive mechanism. It's unclear why the bulk of the experiments were done with very high levels of peroxide, which is really quite severe, and the effects on Yme1 don't seem as robust or specific as Fig1F (compare with Fig 1B, for example where AFG3L2 is also lost, and only at highest concentrations)."*

Our Response to Reviewer #2 Comment #3. In the revised manuscript, we show additional data demonstrating that the addition of antimycin A to cells cultured in galactose-conditioned media leads to the rapid processing of OPA1 (see revised **Figure S3B**), a molecular event indicative of mitochondrial fragmentation. We also show that antimycin A added to these cells does not induce apoptotic signaling or significant OMA1 degradation but does increase OMA1 proteolytic processing to a small conformation of this protease, previously shown to be an active OMA1 protease (Zhang and Song. **2014**. *EMBO Rep*)(see revised **Figure S3B**). Additionally, we include another new panel showing that antimycin A treatment in cells cultured in galactose-containing media results in the rapid depolarization of the mitochondrial inner membrane (directly addressing the reviewer's concerns (see revised **Figure S3C**). We also include additional data showing the addition of antimycin A to cells cultured in glucose-supplemented media does not induce significant YME1L degradation (see revised **Figure S1F**). Collectively, these results show that the addition of antimycin A to cells cultured in galactose-supplemented media leads to selective YME1L degradation, significant depolarization of the mitochondrial inner membrane, the robust activation of OMA1-dependent OPA1 processing, and does not activate apoptotic signaling pathways. We address these new results in the revised manuscript, as below:

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Page 8, Paragraph 2, Line 9: *"Other stresses that induce YME1L degradation such as H2O2 and antimycin A in galactose-cultured cells also do not induce OMA1 degradation (**Figure 3D** & **S3B**), although antimycin A promotes OMA1 cleavage into the active proteolytic fragment. The activation of OMA1 in antimycin-treated cells cultured in galactose is consistent with the depolarization of the mitochondrial membrane in these cells, as shown by reduction in TMRE fluorescence (**Figure S3C**)."*

Page 9, Paragraph 2, Line 3: *"Despite inducing robust YME1L degradation, we observe no caspase-3 activation or PARP cleavage in N2a cells treated with H2O2 (**Figure 4A**) or HEK293T cells cultured in galactose-supplemented media and treated with antimycin A (**Figure S3B**)."*

Page 5, Paragraph 1, Line 11: *"The addition of antimycin A to cells cultured in glucosecontaining media did not influence YME1L protein levels (**Figure S1F**)."*

Additionally, we identify other cell treatments, apart from antimycin A, that similarly result in selective YME1L degradation without impacting AFG3L2. Notably, these include conditions where we selectively depolarize the mitochondrial membrane and deplete cellular ATP levels (see **Figure 3A,B** of the revised manuscript). We now also include additional data using these alternative treatments that induce selective YME1L degradation to demonstrate that OMA1 turnover does not confound the OMA1-dependent degradation of YME1L induced during stress. Specifically, we show that the rapid CCCP-dependent OMA1 autocatalytic turnover is significantly attenuated upon co-administration of 2-deoxyglucose (2-DG; see **Figure 3C** of the revised manuscript). This result directly addresses a concern brought up by the reviewer regarding the relationship between OMA1 and YME1L degradation (see **Our Response to Reviewer #2 Comment #2**). This new data is discussed in the revised manuscript, as below:

Page 8, Paragraph 2, Line 2: "*Thus, we evaluated the relationship between OMA1 and YME1L degradation induced by CCCP in the presence or absence of 2-DG. As observed previously, CCCP induces a reduction in total OMA1 protein levels (**Figure 3C**) [22, 28]. Alternatively, the co-addition of 2-DG with CCCP slows OMA1 degradation and results in the accumulation of a proteolytically processed OMA1 isoform previously shown to retain protease activity (arrows in **Figure 3C**) [28]. The addition of 2-DG alone does not influence OMA1 stability."*

We would also like to address the reviewer's comment regarding our use of '*very high levels of peroxide*'. We primarily use H2O2 at 100-200 μM throughout this manuscript. This is either consistent with or less than that used in the majority of other manuscript describing stress-induced alterations in mitochondrial proteostasis (e.g., Baker *et al.* (**2014**) *EMBO* used 500 μM H2O2 in MEF cells and Stiburek *et al*. (**2012**) *MBoC* used 200 μM in HEK293T cells). While it is clear from our work and the work of many others that different cell types require different levels of H2O2 to induce robust stress, we would like to highlight that our use of H2O2 in this manuscript is consistent with these previously published reports.

Reviewer #2 Comment #4. *"The suggestion is that Oma1 acts along with another unidentified protease, which is certainly logical. However, recent work in both yeast and mammalian systems has hinted towards mechanisms of selective mitophagy (Nat Commun. 2013;4:2789.), or vesicle transport (EMBO J. 2014 Feb 18;33(4):282-95.). These pathways depend on PINK1 and Parkin in mammalian systems. If the authors silence Parkin and PINK1, does Yme1 degradation still occur? Do the authors observe any overlay among the protease cascades and mitophagy? The kinetics of Parkin recruitment (generally 30-90 minutes) would suggest that the protease degradation occurs while mitochondria are undergoing mitophagy (in the peroxide or CCCP treatments at least). In vitro assays suggest the answer is no, but it should be tested formally. Where do the authors think the tipping point is for this protease cascade relative to mitophagy? If Yme1 is cleaved, is this when PINK1 may accumulate, for example? This type of question could be nicely addressed in the discussion to lend context to the non-expert readers."*

Our Response to Reviewer #2 Comment #4. As the reviewer indicates, we show that YME1L degradation can be recapitulated in isolated mitochondria, demonstrating that YME1L degradation occurs independent of proteins such as PINK and PARKIN required for mitophagy

(see **Figures 2A-C, 2F, & S2A-D**). Additionally, we now show that H2O2-induced YME1L degradation occurs in Hela cells – a cell line that lacks active PARKIN protein – demonstrating that YME1L degradation is not directly linked to mitophagy. These results indicate that YME1L degradation occurs independent of mitophagy. We discuss these new results in the revised manuscript, as below:

Page 5, Paragraph 1, Line 4: *"Similar results were observed in all cell lines tested including N2a, HEK293T, and Hela cells – the latter, a cell that lacks PARKIN [18-20], indicating that the loss of YME1L and AFG3L2 is independent of mitophagy (**Figure S1B-D**)."*

Reviewer #2 Comment #5. *"It is unexpected that the megadalton complex shows no sign of disassembly on the way to degradation. It is rather like it just disappeared. Only one time point was examined, 6 hours following 100uM peroxide. Are intermediates caught at earlier time points? If the authors add the protease inhibitors do they observe any accumulation of altered intermediates?"*

Our Response to Reviewer #2 Comment #5. We were also quite surprised that YME1L showed no signs of disassembly in our original BN-PAGE experiments. To address concerns from **Reviewer #2 & 3**, we have adapted our BN-PAGE protocol to use the more sensitive chemiluminescence detection with HRP conjugated secondary, where we can overexpose the images to better visualize the accumulation of smaller oligomeric species. Using this more sensitive detection approach, we do see a modest accumulation of smaller YME1L oligomeric species in OMA1+/+ and OMA1-/- MEFs treated with H2O2 for 3 or 6 h (see revised **Figure 2G**). These smaller oligomers were never observed using the LI-COR detection approach, as we reported in our previous submission. This suggests that there may be some dissociation occurring during the H2O2 treatment. We have appropriately adapted the revised manuscript to highlight this new data obtained using the more sensitive BN-PAGE/immunoblotting protocol, as below:

Page 7, Paragraph 2, Line 1: *"Oxidative stress could promote YME1L degradation through alterations in its oligomeric structure. YME1L exists as an ~1 MDa i-AAA protease complex in mammals, comparable to the Yme1-Mgr1/3 complex in yeast [8, 26, 27]. We monitored the YME1L complexes in OMA1+/+ and OMA1-/- MEFs treated with or without H2O2 using Blue Native polyacrylamide gel electrophoresis (BN-PAGE). YME1L primarily forms an ~1 MDa oligomeric complex in both OMA1+/+ and OMA1-/- MEFs, with a small population of smaller YME1L complexes between 720 kDa and 242 kDa (**Figure 2G**). The addition of H2O2 increases the populations of these smaller complexes, indicating that H2O2 affects YME1L complex stability **(Figure 2G**). This suggests that YME1L degradation may proceed through dissociation of the YME1L oligomer, although further mechanistic studies are required to define a specific role for YME1L dissociation in this process."*

Reviewer #2 Comment #6. *"The authors should directly address the potential role of LonP, MPPs or the Clp proteases in the turnover of Yme1. They should really all be screened as was done for the cleavage of Opa1 and PINK1 over the last number of years by various authors."*

Our Response to Reviewer #2 Comment #6. As suggested by the reviewer, we have expanded our analysis of the mitochondrial protease(s) responsible for stress-induced YME1L degradation (for further discussion, please see **Our Response to Reviewer #1 Comment #4**). Briefly, we screened a series of protease inhibitors for their ability to inhibit YME1L degradation in isolated mitochondria (see revised **Figure S2D**). Using this approach, we found that only inhibitors of metalloproteases with active sites oriented to the IMS are sufficient to attenuate in vitro YME1L degradation. These results indicate that LON, CLPP, and MPP (whose active sites are oriented to the matrix) are not involved in this process. These new results are discussed in the revised manuscript as below:

Page 6, Paragraph 2, Line 1: *"YME1L degradation in isolated mitochondria incubated in the absence of ATP is also inhibited by the zinc chelator o-phenanthroline (o-phe) and the divalent cation chelator EDTA (**Figure 2C**). Inhibitors of serine, cysteine, and aspartic acid proteases did not inhibit YME1L degradation in isolated mitochondria (**Figure S2D**). This suggests that*

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YME1L degradation involves the activity of an ATP-independent zinc metalloprotease. Specifically, a metalloprotease with an active site oriented towards the intermembrane space (IMS), as EDTA cannot cross the inner mitochondrial membrane and only inhibits metalloproteases with IMS-oriented active sites [21]."

Reviewer #2 Comment #7. *"There is discussion about a potential accumulation of oxidized proteins after the time when Yme1 is degraded. It is important to test this, which can be done by silencing Oma1 to protect against Yme1 loss, and perform oxyblot experiments to monitor the accumulation of oxidized proteins in cells where Yme1 is lost (control, with stress), or not (Oma1-/-)."*

Our Response to Reviewer #2 Comment #7. The experiment suggested by the reviewer is extremely difficult because OMA1-/- cells do not completely inhibit stress-induced YME1L degradation (see revised **Figure 2D,E**) and the methods available to measure oxidatively modified inner membrane proteins (e.g., Oxyblot of mitochondrial membrane fractions) are not sufficiently sensitive to accurately quantify differences in intracellular populations of oxidatively-modified mitochondrial inner membrane proteins under the oxidative stress conditions required to induce YME1L degradation (although this approach is sufficient for more qualitative analyses).
Thus, we focused our efforts in the revised manuscript to define the specific impact of stress-induced YME1L degradation on the capacity for cells to regulate mitochondrial inner membrane proteostasis through stress-regulated YME1L functions. Specifically, we focused on evaluating the impact of stress-induced YME1L degradation on the protective, YME1L-dependent degradation of the core TIM23 subunit Tim17A. Following the stability of the established YME1L substrate Tim17A provides the advantage to accurately monitor the impact of stresses that induce YME1L degradation on a specific activity of YME1L that can be initiated by adding stresses that induce eIF2a phosphorylation or inhibit translation and then sensitively followed by quantitative immunoblotting. We show that treating cells with H2O2 induces eIF2a phosphorylation, but does not lead to eIF2a phosphorylation-dependent degradation of Tim17A (see revised **Figure 4C**). Furthermore, we show that pretreating cells with H2O2 significantly impairs YME1L-dependent Tim17A degradation induced by cycloheximide treatment (see revised **Figure 4D**). We also include new data to show that genetic depletion of *TIM17A* (mimicking YME1L-dependent regulation of this mitochondrial protein import factor) increases cellular survival in response to H2O2 treatment (see revised **Figure 4G**). This data indicates that suppression of YME1L-dependent Tim17A degradation afforded by H2O2-induced YME1L degradation decreases cellular capacity to regulate mitochondrial proteostasis. Collectively, these results using a model stress-regulated YME1L substrate report on YME1L proteolytic activity and demonstrate that the stress-induced loss of YME1L is detrimental to cellular capacity to regulate mitochondrial proteostasis in response to oxidative insult. We have adapted the text to better indicate our use of Tim17A as a model YME1L substrate and to highlight our new data indicated above. These changes are included in the revised manuscript, as below:

Page 9, Paragraph 3, Line 1: "*Alternatively, YME1L degradation should decrease the capacity for cells to regulate inner membrane proteostasis. We monitored the impact of oxidative-stress dependent YME1L degradation on mitochondrial inner membrane proteostasis regulation through the stress-regulated degradation of the YME1L substrate Tim17A [30]. Tim17A is a core TIM23 subunit that is rapidly degraded by YME1L in response to stress-regulated eIF2α phosphorylation-dependent translational attenuation [30]. YME1L-dependent Tim17A degradation is a protective mechanism to attenuate mitochondrial protein import and increase cellular viability in response to stress [30]. Despite inducing eIF2α phosphorylation [31], Tim17A is not degraded in H2O2-treated SHSY5Y cells (**Figure 4C**)."*

Page 10, Paragraph 1, Line 5: *"Pretreatment with H2O2 attenuates CHX-dependent Tim17A degradation, consistent with H2O2-induced YME1L degradation inhibiting Tim17A regulation (**Figure 4D**). H2O2-dependent YME1L degradation is not affected by TIM17A-depletion, showing that YME1L degradation occurs upstream of Tim17A (**Figure S4B**). Importantly, the addition of H2O2 to isolated mitochondria does not inhibit the YME1L-mediated, ATP-dependent degradation of Tim17A (**Figure S4C**). This shows that H2O2 does not antagonize the ability for*

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YME1L to degrade Tim17A, but instead reflects the predicted reduction in YME1L activity in cells treated with H2O2. Collectively, these results indicate that H2O2 inhibits YME1L proteolytic activity, compromising the capacity for cells to regulate mitochondrial inner membrane proteostasis during oxidative stress."

*Page 10, Paragraph 2, Line 5: "This suggests that the loss of YME1L-dependent regulation of mitochondrial proteostasis (such as Tim17A degradation) sensitizes cells to oxidative insult. Consistent with this prediction, depletion of TIM17A in HEK293T cells – mimicking stressregulated Tim17A degradation – increases cellular viability in response to H2O2, suggesting that inhibition of YME1L-dependent Tim17A degradation reduces cellular capacity to regulate mitochondria in response to oxidative insult (**Figure 4G**)."*

REVIEWER #3.

Reviewer #3 General Comments: *"Overall, an interesting paper that addresses an important question regarding mitochondrial proteolysis regulation. The authors report a novel mechanism of regulation of YME1L stability, through oxidative stress combined with nucleotide depletion. They show that OMA1 is a major protease involved in YME1L degradation and their results suggest that this degradation of YME1L is involved in cell death resulting from oxidative stress. This final point, which demonstrates the biological relevance of this study is important and is not well supported by the data. Further experimental support is needed to strengthen this study. Specifically the authors should address the following:"*

Our Response to Reviewer #3 General Comments. We thank the reviewer for the careful reading of our manuscript and for providing constructive comments to improve our manuscript. We address all of the reviewer's important comments in our point-by-point response included below.

Reviewer #3 Comment #1. *"The major concern is the lack of solid experimental data demonstrating the biological relevance. The authors should confirm that YME1L degradation is important in cell sensitivity in response to oxidative stress, by showing that preventing the degradation of YME1L can enhance cell survival in response to stress, and this should be demonstrated several ways in more than one cell type. Does over expression of YME1L improve survival after oxidative stress"*

Our Response to Reviewer #3 Comment #1. In the revised manuscript, we provide additional new data to address the point brought up by the reviewer. As discussed above in **Our Response to Reviewer #1 Comment #1**, we now show that the degradation of YME1L does not directly result from apoptotic signaling, indicating that YME1L degradation is not directly linked to commitment to programmed cellular death. Similarly, as discussed in **Our Response to Reviewer #2 Comment #1**, we now show that YME1L protein levels can recover following a 24 h recovery treatment following H2O2 treatment, directly demonstrating YME1L degradation does not solely lead to cell death. Additionally, we now provide new results describing the relationship between stress-induced degradation of YME1L and OMA1, revealing potential functional interplay between stress-mediated alterations in mitochondrial proteolytic activity that can sensitively adapt mitochondrial proteostasis regulation in response to distinct pathologic insults (see **Our Response to Reviewer #2 Comment #2**). Furthermore, we provide additional data to show that YME1L degradation is induced independent of mitophagy (see **Our Response to Reviewer #2 Comment #4**.

Unfortunately, the experiments suggested by the reviewer to demonstrate the biological significance of YME1L degradation are currently not possible. We can attenuate YME1L degradation in OMA1-/- MEFs, but stress-induced YME1L degradation is not completely inhibited in these cells. This significantly compromises our ability to evaluate stress-induced YME1L degradation and the impact of this degradation on mitochondrial function and/or cell viability (see **Our Response to Reviewer #2 Comment #7**). OMA1-/- MEFs are also known to show significant stress resistance to a variety of stresses (including those that do not induce

YME1L degradation), indicating that it will be extremely difficult to directly define the specific impact of YME1L degradation vs. other OMA1 functions on mitochondrial dynamics/proteostasis regulation in response to pathologic insults. Finally, we are currently unable to overexpress tagged YME1L constructs to high levels in cell culture models, preventing experiments where we could inhibit YME1L degradation by overexpressing a YME1L mutant resistant to this process. We are currently developing new genetic and chemical biologic approaches to control YME1L stability and/or activity that will be very useful to address the specific impact of stress-induced YME1L degradation on mitochondrial proteostasis and function, as suggested by the reviewer, but these are beyond the scope of this initial report.

To address the reviewer's comment regarding the biological impact of YME1L degradation on mitochondrial proteostasis regulation, in the revised manuscript, we specifically focused on the protective, stress-induced degradation of the YME1L model substrate Tim17A. As discussed above in **Our Response to Reviewer #2 Comment #7**, focusing on Tim17A regulation offers significant advantages that allow us to demonstrate that stress-induced YME1L degradation decreases YME1L proteolytic activity and attenuates cellular capacity to regulate mitochondrial inner membrane proteostasis. As shown in **Figure 4C,D** of the revised manuscript, we demonstrate that H2O2 attenuates the protective degradation of Tim17A by YME1L. Furthermore, we now include additional data showing that mimicking YME1L-dependent Tim17A degradation increases cellular viability when cells are challenged with oxidative insults. We also now show that *TIM17A*depleted cells have increased resistance to H2O2 treatments (see revised **Figure 4G**) – a stress that prevents YME1L-dependent Tim17A regulation. This suggests that stress-induced YME1L degradation reduces the cell's capacity to regulate mitochondrial proteostasis through Tim17A degradation, thereby decreasing cellular stress resistance. These data are consistent with our model wherein YME1L degradation impairs cellular capacity to regulate mitochondrial proteostasis and sensitizes cells to subsequent oxidative insults. We believe that these new results better highlight the biologic significance of YME1L degradation in this initial report and are consistent with the level of biologic impact included with other initial reports of stress-regulated remodeling of mitochondrial proteostasis pathways in the literature. We discuss these new results in the revised manuscript, as below:

*Page 10, Paragraph 2, Line 5: "This suggests that the loss of YME1L-dependent regulation of mitochondrial proteostasis (such as Tim17A degradation) sensitizes cells to oxidative insult. Consistent with this prediction, depletion of TIM17A in HEK293T cells – mimicking stressregulated Tim17A degradation – increases cellular viability in response to H2O2, suggesting that inhibition of YME1L-dependent Tim17A degradation reduces cellular capacity to regulate mitochondria in response to oxidative insult (**Figure 4G**)."*

Reviewer #3 Comment #2. *"Blue native gel is poor quality (Fig.2G) - better gel should be provided."*

Our Response to Reviewer #3 Comment #2. As suggested by the reviewer, we have further repeated this experiment. In these new experiments, we employed a more sensitive HRP detection method (as opposed to the Li-ICOR detection used previously). Using this more sensitive approach, we do observe some YME1L dissociation into smaller oligomers that occurs in both OMA1+/+ and OMA1-/- MEFs treated with H2O2. This dissociation was never observed using the LI-COR immunoblotting approach (See **Our Response to Reviewer #2 Comment #5)**. We appreciate the suggestion of the reviewer to improve our BNPAGE/ immunoblotting analysis of this point, as we feel that it has led to an important potential discovery in the mechanism of YME1L degradation. We address these new data in the revised manuscript, as below:

Page 7, Paragraph 2, Line 1: *"Oxidative stress could promote YME1L degradation through alterations in its oligomeric structure. YME1L exists as an ~1 MDa i-AAA protease complex in mammals, comparable to the Yme1-Mgr1/3 complex in yeast [8, 26, 27]. We monitored the YME1L complexes in OMA1+/+ and OMA1-/- MEFs treated with or without H2O2 using Blue Native polyacrylamide gel electrophoresis (BN-PAGE). YME1L primarily forms an ~1 MDa*

*oligomeric complex in both OMA1+/+ and OMA1-/- MEFs, with a small population of smaller YME1L complexes between 720 kDa and 242 kDa (**Figure 2G**). The addition of H2O2 increases the populations of these smaller complexes, indicating that H2O2 affects YME1L complex stability **(Figure 2G**). This suggests that YME1L degradation may proceed through dissociation of the YME1L oligomer, although further mechanistic studies are required to define a specific role for YME1L dissociation in this process."*

Reviewer #3 Comment #3. *"The data does not fully support the model shown in Fig.3G indicating that YME1L is destabilized, infact this model is inconsistent with the data in Fig. 2G. Model should be amended appropriately."*

Our Response to Reviewer #3 Comment #3. In the revised manuscript we present a modified figure to depict the working model for YME1L degradation (see revised **Figure 3E**). In particular, based on the data presented in the revised **Figure 2G** we have altered the text in the model to indicate that YME1L containing complexes appear to change conformation and increase the population of smaller oligomer complexes in response to stress. In addition, we have clarified the figure to indicate that through damage or depolarization, the mitochondria inner membrane potential ($\Delta\psi$) is depleted to activate OMA1. Likewise, we have added a second circle labeled with a question mark to indicate the possible involvement of another protease in the turnover of YME1L. We feel that this figure simply and accurately depicts the current working model for the process of YME1L degradation during stress.

2nd Editorial Decision 21 October 2014

Thank you for your patience while we have reviewed your revised manuscript. I apologize for the delay in getting back to you. While all three reviewers appreciate the that the revised version has been strengthened, referee 1 still raises concerns about some of the data, mainly with regard to apparent inconsistencies about the effects of loss of different TIM components on viability. Upon further discussions with one of the other reviewers, I came to the conclusion that it would suffice if you more clearly discussed these results and offered some hypotheses on how to explain these discrepancies.

This is to say that this is a 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once these additional clarifications/discussions have been added. Please don't worry about length restrictions in this case, as we can be flexible.

If all remaining corrections have been attended to, you will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports.

REFEREE REPORTS:

Referee #1:

The authors have addressed several points of criticism in the revised manuscript and provide now evidence that the observed degradation of YME1L is not due to apoptosis, one of my major concerns. Overall, the experiments are well-performed and the complex regulation of YME1L stability under different stress conditions and apoptosis is intriguing. However, the manuscript still falls short to provide insight into the physiological relevance of stress-induced YME1L turnover. I agree with the authors that the loss of YME1L activity will increase the cellular sensitivity to stress (consistent with previous knockdown/knockout studies), but what is it good for then? The authors should discuss their findings in light of the role of YME1L for regulating mitochondrial dynamics and cristae formation.

I am also confused about the link to apoptosis. YME1L deficient cells are more susceptible to apoptosis, which has been attributed before to deficient cristae morphogenesis. The authors have described previously an additional possibility, the YME1L-dependent degradation of TIMM17A as a protective mechanism. However, the loss of TIMM17 (e.g. RNAi-mediated downregulation or YME1L-mediated proteolysis) which was previously shown to reduce protein import (Rainboldt et al 2013), would be predicted to have a similar effect as the loss of TIMM23, which was shown to increase sensitivity to cell death. The authors show the opposite: TIMM17A depletion increases apoptotic resistance (Fig. 4G). While this is consistent with the phenotype observed in OMA1-/- cells, it is inconsistent with our knowledge of TIMM23 and cell death.

Apparent inconsistencies notwithstanding, the biological relevance of this study remains highly speculative, although the implications to mitochondrial quality control, mitophagy, and protein import may be of great relevance.

Referee #2:

In the previous submission the authors had made an interesting and important observation concerning the selective degradation of Yme1. However I was concerned about the functional contribution of its degradation to the death program, as well as the relationship of this cleavage event with respect to the other mitochondrial proteases and proteolytic events. The emerging paradigm of the protease cascade is complex and challenging, but the authors have now provided compelling new data that have addressed each of my concerns with thoughtful and informative new experiments. Ultimately they place the Oma1 dependent degradation of Yme1 during cellular stress as a critical event that increases the sensitivity to cell death. I applaud their efforts in this revision and have no remaining concerns. This work will be an important step in our understanding of the process of mitochondrial proteostasis, which is of increasing importance in many pathological conditions.

Referee #3:

The authors have made a major effort to address most of the concerns raised. They were still unable to perform rescue experiments and were not able to over express YME1L in cells, but they are currently developing new genetic approaches to deal with this. The BN-PAGE quality has been improved and the model has been amended to more accurately reflect their data.

Thus, the authors have made significant revisions to strengthen this paper. This will be a nice contribution to the field.

2nd Revision - authors' response 30 October 2014

RESPONSE TO EDITORIAL AND REVIEWER CONCERNS

EDITOR GENERAL COMMENTS. *"Thank you for your patience while we have reviewed your revised manuscript. I apologize for the delay in getting back to you. While all three reviewers appreciate the that the revised version has been strengthened, referee 1 still raises concerns about some of the data, mainly with regard to apparent inconsistencies about the effects of loss of different TIM components on viability. Upon further discussions with one of the other reviewers, I came to the conclusion that it would suffice if you more clearly discussed these results and offered some hypotheses on how to explain these discrepancies."*

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Our Response to Editor General Comments. We thank the editor for '*accepting our manuscript in principle*' and for the opportunity to clarify our results regarding the differential effects of Tim17A and Tim23 degradation in protecting cells from pathologic insults. We directly address this point brought up by Reviewer #1 in **Our Response to Reviewer #1** discussed below. In addition, we prepared a short summary and 3 bullet points describing our work in the revised submission, as requested.

OUR RESPONSE TO REVIEWER #1.

Reviewer #1 Comment #1. "*The authors have addressed several points of criticism in the revised manuscript and provide now evidence that the observed degradation of YME1L is not due to apoptosis, one of my major concerns. Overall, the experiments are well-performed and the complex regulation of YME1L stability under different stress conditions and apoptosis is intriguing. However, the manuscript still falls short to provide insight into the physiological relevance of stress-induced YME1L turnover. I agree with the authors that the loss of YME1L activity will increase the cellular sensitivity to stress (consistent with previous knockdown/knockout studies), but what is it good for then? The authors should discuss their findings in light of the role of YME1L for regulating mitochondrial dynamics and cristae formation.*

Our Response to Reviewer #1 Comment #1. We appreciate the reviewer's comments about our manuscript. With respect the reviewer's comment regarding '*what is [YME1L degradation] good for'*, we would like to highlight that our results indicate that oxidative-stress induced YME1L degradation contributes to the mitochondrial dysfunction associated with the pathophysiology of many human diseases. Thus, our results do not indicate that YME1L degradation as a beneficial cellular mechanism to adapt mitochondrial inner membrane proteostasis during stress, but instead, our results indicate that YME1L degradation is a pathologic effect of oxidative stress that decreases cellular capacity to regulate mitochondrial inner membrane proteostasis, rendering mitochondria and thus cells more sensitive to oxidative insult. While it remains possible that YME1L degradation could be beneficial under certain conditions (e.g., segregation of terminally damaged mitochondria), our results are most consistent with a pathologic role for YME1L degradation during oxidative stress. We have adapted our text in this second revision to further highlight this point, as below:

> Page 8, Paragraph 2, Line 13: *"These results show that OMA1 and YME1L degradation are differentially sensitive to cellular stresses, potentially yielding distinctive mitochondrial inner membrane proteolytic activities in response to specific pathologic insults."*
>
> Page 11, Paragraph 3, Line 8: *"While our results show that oxidative stress-induced YME1L degradation is a mechanism that can contribute to the pathologic mitochondrial dysfunction involved in human diseases, YME1L degradation could also play a regulatory role in adapting mitochondrial function during conditions of stress. For example, it was recently shown that YME1L activity is needed for the de novo cleavage of OPA1 to mediate fusion events [10]. Therefore, YME1L degradation may also provide a mechanism to sequester terminally damaged mitochondria away from the healthy pool thereby restricting them to clearance via mitophagy, although additional studies will be required to define these potentially protective mechanisms of YME1L degradation in mitochondrial regulation."*

Reviewer #1 Comment #2 "*I am also confused about the link to apoptosis. YME1L deficient cells are more susceptible to apoptosis, which has been attributed before to deficient cristae morphogenesis. The authors have described previously an additional possibility, the YME1L-dependent degradation of TIMM17A as a protective mechanism.*

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However, the loss of TIMM17 (e.g. RNAi-mediated downregulation or YME1L-mediated proteolysis) which was previously shown to reduce protein import (Rainboldt et al 2013), would be predicted to have a similar effect as the loss of TIMM23, which was shown to increase sensitivity to cell death. The authors show the opposite: TIMM17A depletion increases apoptotic resistance (Fig. 4G). While this is consistent with the phenotype observed in OMA1-/- cells, it is inconsistent with our knowledge of TIMM23 and cell death.

Apparent inconsistencies notwithstanding, the biological relevance of this study remains highly speculative, although the implications to mitochondrial quality control, mitophagy, and protein import may be of great relevance."

Our Response to Reviewer #1 General Comments. We thank the reviewer for the careful reading of our revised manuscript. In the second revision, we now explicitly discuss the differences in cellular viability afforded by reducing Tim17A or Tim23 protein levels. Briefly, mammalian TIM23 complexes contain two distinct core complexes defined by the presence of one of the two mammalian Tim17 proteins Tim17A or Tim17B. This is distinct from yeast complexes that encode only a single Tim17 protein and thus exist as a single TIM23 complex consisting of a core Tim23-Tim17 interaction. Our previous work shows that Tim17A, but not Tim17B, is rapidly degraded in a YME1L-dependent process downstream of stress-regulated eIF2a phosphorylation (Rainbolt et al 2013). The selective degradation of Tim17A reduces the population of TIM23 complexes containing a core Tim17A subunit, but does not impact TIM23 complexes containing a core Tim17B subunit (Rainbolt et al 2013). Thus, the effect of reducing Tim17A levels is an attenuation, but not complete inhibition, of mitochondrial protein import, as we described previously (Rainbolt et al 2013). Alternatively, the core TIM23 subunit Tim23 is degraded in response to apoptotic stimuli in the presence of caspase inhibitors (Goemans et al 2008). Since Tim23 is found in *all* mammalian TIM23 complexes (Bauer et al 1999), the reduction in Tim23 afforded by this degradation will *completely* inhibit TIM23-dependent protein import, as described previously (Goemans et al 2008). Thus, reducing Tim17A and Tim23 will differentially affect mitochondrial protein import and thus cellular viability. Consistent with this prediction, we showed previously and again in this manuscript that RNAi-depletion of *TIM17A* is protective against cellular stress (Rainbolt et al 2013). Alternatively, Tim23 degradation appears to promote caspase-independent death in response to apoptotic stimuli (Goemans et al 2008). Additionally, this effect is further evident as *TIM23*-depletion stops cellular proliferation (Goemans et al 2008), while *TIM17A* depletion has no effect on cellular growth (Rainbolt et al 2013 and in this manuscript). We discuss this point explicitly in the revised manuscript, as below:

> Page 9, Paragraph 3, Line 1: *"Alternatively, YME1L degradation could decrease the capacity for cells to regulate inner membrane proteostasis. To explore this potential consequence of YME1L degradation, we monitored the impact of oxidative stress on YME1L mediated regulation of the TIM23 mitochondrial protein import complex [30]. Mammalian TIM23 forms two exclusive complexes containing distinct core interactions between the subunit Tim23 and one of the two mammalian paralogs of yeast Tim17, Tim17A or Tim17B [31]. Previous work showed that Tim17A is a stress-regulated TIM23 subunit that is rapidly degraded by YME1L in response to eIF2α phosphorylation-dependent translational attenuation [30]. However, Tim17B is not subject to this regulation. Thus, YME1L-mediated degradation of Tim17A reduces the population of active TIM23 complexes containing a core Tim23-Tim17A interaction without impacting TIM23 complexes containing a core Tim23-Tim17B interaction [30]. This provides a mechanism for cells to sensitively attenuate, but not completely inhibit, TIM23-dependent protein import in response to pathologic insult [30]. The attenuation in mitochondrial protein import afforded by Tim17A degradation is predicted to promote mitochondrial proteostasis through mechanisms such as reducing the population of newly-synthesized unfolded proteins entering mitochondrial during stress and by promoting transcriptional remodeling of mitochondrial proteostasis pathways [30, 32]. Consistent with a protective role for Tim17A degradation, reducing Tim17A levels increases cellular viability in response to mitochondrial insults such*

as paraquat [30]. The benefits attributed to YME1L-dependent Tim17A degradation are in contrast to those observed for the degradation of Tim23, the core channel forming subunit of TIM23 complexes, which is observed during cell death in the presence of caspase inhibitors [33]. Since Tim23 is an essential subunit for all TIM23 complexes, reductions in Tim23 will decrease the cellular populations of all active TIM23 complexes, completely inhibiting TIM23-dependent mitochondrial protein import [33]. Consistent with the differential impacts of reducing Tim17A or Tim23 on mitochondrial protein import, RNAi-depletion of TIM23 inhibits cellular proliferation, while TIM17A depletion does not impact cellular growth [30, 33]. These results serve to further highlight the protective role for selective, YME1L-dependent Tim17A degradation in regulating TIM23-dependent mitochondrial protein import, promoting mitochondrial proteostasis and increasing cellular viability in response to stress [30].”

3rd Editorial Decision 31 October 2014

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. Please consider us again in the future for your most exciting work.