Ablation of mitochondrial DNA results in widespread remodeling of the mitochondrial complexome

Sergio Guerrero-Castillo, Joeri van Strien, Ulrich Brandt, and Susanne Arnold
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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)
Dear Dr. Brandt,

Thank you for submitting your study entitled "Ablation of mitochondrial DNA results in widespread remodeling of the mitochondrial complexome". The manuscript has been assessed by three reviewers, whose reports are enclosed below.

As you will see, the referees find your study a potentially valuable resource article. However, they also raise some points that need to be addressed before they can support publication here.

Given the interest in your complexome profiling as a resource article, I am pleased to invite submission of a manuscript revised as indicated in the reports attached herein. In addition, I would ask you to emphasize the "resource" character of your study in the abstract and discussion sections of the manuscript.

I should also add that it is our policy to allow only a single round of major revision. Therefore, acceptance of your manuscript will depend on the completeness of your responses in this revised version.

We generally grant three months as standard revision time. As we are aware that many laboratories cannot function at full capacity owing to the COVID-19 pandemic, we may relax this deadline. Also, we have decided to apply our 'scooping protection policy' to the time span required for you to fully revise your manuscript and address the experimental issues highlighted herein. Nevertheless, please inform us as soon as a paper with related content is published elsewhere.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Yours sincerely,

Elisabetta Argenzio, PhD
Editor
The EMBO Journal
Referee #1:

The mitochondrial DNA encodes for core components of the respiratory chain complexes and the F1FO ATP synthase. Guerrero-Castillo and colleagues investigated how loss of the mitochondrial DNA in rho0 cells alters the mitochondrial proteome using mass spectrometry. In addition, the authors provide a comprehensive overview about changes in mitochondrial protein complexes using blue native electrophoresis-based complexome studies. They found major changes in the mitochondrial proteome and complexome upon loss of mitochondrial DNA. Respiratory chain complexes and translation machineries dissociates in rho0 cells as expected. However, subassemblies accumulate that could represent intermediates during formation of the mature complex. Loss of mitochondrial DNA also remodels the carrier proteins, TCA cycle enzymes and affect the abundance and organization of protein translocases in both membranes.

Overall, the study is highly interesting and of excellent quality. The impressive changes of both the proteome and complexome are exciting and provide important insights into mitochondrial biology. The presented data sets are a rich resource for mitochondrial research. The findings are highly interesting for the broad readership of EMBO J. I have a few minor suggestions the authors should address in the revision.

1. Figure 1 is important, but the presentation should be improved. In the current version, it is not possible to see which proteins are affected in rho0 mitochondria. I recommend to show a cartoon of a few selected mitochondrial protein machineries (respiratory chain complexes, MICOS, ribosomes, protein translocases etc.), indicating which protein machineries/components are up- and down-regulated. The authors may also indicate in Figure 1B, which respiratory chain subunits are up-regulated.

2. Figure 6: The distributions of several TOM, TIM23 and TIM22 subunits are combined in TOM avg, TIM23 avg and TIM22 avg. The authors should also provide the plots for single subunits in the supplement. It should be clearly stated in the text that TOMM34 is a cytosolic factor and not a TOM subunit.

3. The authors showed that TOMM40L, a TOMM40-related protein, forms a complex that is smaller than the TOM complex. Does TOM40L associate with other TOM subunits?
5. In Figure 6D the authors showed a co-migration of TIMM21 with YME1L, PHB, ROMO-1 and DNAJ proteins. This is highly interesting since TIMM21 was established as a component of the TIM23 complex. How large is the TIMM21 population that co-migrates with YME1L compared to the population that co-migrates with TIMM23? MAGMAS is an interaction partner of DNAJC proteins at the TIM23 complex. Is MAGMAS also part of this complex?

Referee #2:

The authors have examined the mitochondrial complexome in cells lacking mtDNA (Rho zero cells) and in the parent cell line from which they were derived (143B osteosarcoma). They identified nearly 1000 mitochondrial proteins in this analysis, which represents a substantial proportion of the mitochondrial proteome in the Mitocarta database. This group is expert at complexome analysis and the data presented here are of high quality. The paper is entirely descriptive and therefore is more appropriately considered a resource for the mitochondrial community. There are, however, some rather unexpected observations, which could inform future functional studies. I have no concerns about the data quality or the presentation.

Referee #3:

The paper by Guerrrero-Castillo et al focuses in the analysis of the mitochondria complexes in rho0 cells. The main method is complexome profiling, which is the most appropriate method, demonstrated by several groups and studies, to analyze global changes in stress conditions and certain mutations in mitochondria. The method in itself is interesting because it provides insight and a large amount of useful information about the reorganization of the mitochondrial proteome in human rho0 cells and on what proteins/complexes/subcomplexes are stable in the absence of the mtDNA-encoded elements and the genome itself. Although I think this paper can be used as a useful resource for future studies, it lacks mainly in functional characterisation of the changes observed and any mechanistic insight. It might be more appropriate to be published as a Resource paper in journals that are more appropriate for this type of work.

Major points:

- The paper is fundamentally descriptive and does not provide any clue about what on the functional implications of these reorganizations. Some key observations (for example lack of effects on MICOS subunits) are puzzling, and yet no effort has been made to address these from a mechanistic point of view. More general point which a non-specialist would wonder: why do rho0 cells need mitochondria at all? Some discussion on this in view of the data would be needed.
- The quantitative data are based on label-free peptide intensity quantifications, which depend on many technical variables. In fact, there seems to be a lot of variability among the four replicates both in rho0 and WT cells. It might be convenient to validate these quantifications using an independent method, for example WB and immunodetection of representative proteins.
- On page 4 when talking about the DNA and RNA polymerases etc, the solubilization and separation methods used for BNGE most probably eliminate the nucleic acids from the samples, so it is difficult to say how much of these factors are actually bound to DNA and RNA in the WT cells.
- There are some points that should be discussed in the context of previous observations:
In page 4, Oxidative phosphorylation section, the fact that there is an F1 subcomplex of complex V in the rho0 cells has been known for quite some time now. In fact, its presence was deemed necessary to maintain the membrane potential through ATP hydrolysis (Buchet, K., and Godinot, C. (1998). Functional F1-ATPase essential in maintaining growth and membrane potential of human mitochondrial DNA-depleted rho degrees cells. J Biol Chem 273, 22983-22989.) (Appleby, R.D., Porteous, W.K., Hughes, G., James, A.M., Shannon, D., Wei, Y.H., and Murphy, M.P. (1999). Quantitation and origin of the mitochondrial membrane potential in human cells lacking mitochondrial DNA. Eur J Biochem 262, 108-116.). This is not really compatible with the explanation the authors give for the reason why IF1 is found bound to this species in the rho0 cells, please discuss. Also, IF1 seems to be bound to the intermediates of CV during their assembly before the incorporation of the mtDNA-encoded subunits (He, J., Ford, H.C., Carroll, J., Douglas, C., Gonzales, E., Ding, S., Feamley, I.M., and Walker, J.E. (2018). Assembly of the membrane domain of ATP synthase in human mitochondria. Proceedings of the National Academy of Sciences of the United States of America 115, 2988-2993.)

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In page 9, it is really striking that there are no changes in the MICOS complexes in the rho0 cells. How could the distribution of these complexes in the altered cristae of the rho0 cells be investigated further?

Minor points:
- It would be good to have a graphical representation in Figure 8 of what OXPHOS subcomplexes accumulate in the rho0 cells.
- In page 10, last paragraph, it should be "OMA1" instead of "OMA2".
- The authors mention during the results and discussion sections the fact that their datasets contain information about cytosolic components. However, how reliable can the quantifications be if there are differences in the presence of these 'contaminants' in the different mitochondrial fraction.
preparations used in the analysis. I think this is not a very well documented point and should not be part of this paper as it dilutes the message and is open to many interpretations.
Point-by-point response to reviewers’ comments:

Referee #1:
The mitochondrial DNA encodes for core components of the respiratory chain complexes and the F1FO ATP synthase. Guerrero-Castillo and colleagues investigated how loss of the mitochondrial DNA in rho0 cells alters the mitochondrial proteome using mass spectrometry. In addition, the authors provide a comprehensive overview about changes in mitochondrial protein complexes using blue native electrophoresis-based complexome studies. They found major changes in the mitochondrial proteome and complexome upon loss of mitochondrial DNA. Respiratory chain complexes and translation machineries dissociates in rho0 cells as expected. However, subassemblies accumulate that could represent intermediates during formation of the mature complex. Loss of mitochondrial DNA also remodels the carrier proteins, TCA cycle enzymes and affect the abundance and organization of protein translocases in both membranes.

Overall, the study is highly interesting and of excellent quality. The impressive changes of both the proteome and complexome are exciting and provide important insights into mitochondrial biology. The presented data sets are a rich resource for mitochondrial research. The findings are highly interesting for the broad readership of EMBO J. I have a few minor suggestions the authors should address in the revision.

We thank the reviewer for these favorable comments and the careful evaluation of our manuscript.

1. Figure 1 is important, but the presentation should be improved. In the current version, it is not possible to see which proteins are affected in rho0 mitochondria. I recommend to show a cartoon of a few selected mitochondrial protein machineries (respiratory chain complexes, MICOS, ribosomes, protein translocases etc.), indicating which protein machineries/components are up- and down-regulated. The authors may also indicate in Figure 1B, which respiratory chain subunits are up-regulated.

We appreciate these valuable suggestions. As proposed, changes in abundance of the components of some mitochondrial protein machineries are now highlighted in color in Figure 1A and selected subunits are marked in Figure 1B.

2. Figure 6: The distributions of several TOM, TIM23 and TIM22 subunits are combined in TOM avg, TIM23 avg and TIM22 avg. The authors should also provide the plots for single subunits in the supplement. It should be clearly stated in the text that TOMM34 is a cytosolic factor and not a TOM subunit.

As requested, the plots for the single subunits are now presented in Expanded View Figure EV5. To clearly indicate that TOMM34 is a cytosolic factor, we amended lines 310-313 as follows: “TOMM70 that was and TOMM34 that was not changed in abundance by mtDNA ablation (Dataset EV1), are required for recruiting mitochondrial carriers to the import pathway, the latter by acting as a cytosolic co-chaperone of Hsp70/Hsp90 (Faou & Hoogenraad, 2012), but were not found associated with the TOM40 complex (Figure 6B).”

3. The authors showed that TOMM40L, a TOMM40-related protein, forms a complex that is smaller than the TOM complex. Does TOMM40L associate with other TOM subunits?

We found no indication for an association of TOMM40L with other TOM subunits and have added the following sentence to indicate this fact (lines 315-318): “The apparent mass of ~300 kDa of the TOMM40L complex would be consistent with the formation of an octamer, but it cannot be
excluded that it contains additional as yet unidentified proteins. However, no components of the TOM machinery were found to co-migrate with TOMM40L (Dataset EV2)."

4. In Figure 6D the authors showed a co-migration of TIMM21 with YME1L, PHB, ROMO-1 and DNAJ proteins. This is highly interesting since TIMM21 was established as a component of the TIM23 complex. How large is the TIMM21 population that comigrates with YME1L compared to the population that co-migrates with TIMM23? MAGMAS is an interaction partner of DNAJC proteins at the TIM23 complex. Is MAGMAS also part of this complex?

The reviewer raises an interesting question. In control mitochondria, we analyzed the relative amounts of TIMM21 in the 1.3 MDa complex and the TIM23 machinery and added this information in line 341-346: "In control cells, ~18% of TIMM21 was found in this mass range. In ρ0 mitochondria, the amount of this complex was halved (Figure 6D) and now corresponded to just ~5% of TIMM21, since its overall amount was increased 1.7-fold (Dataset EV1). Both in 143B and ρ0 cells, about one third of TIMM21 co-migrated with the TIMM23 indicating that its increase in abundance largely reflected the doubling of the amount of the TIM23 machinery caused by ablation of mtDNA (Figure 6C)."

Unfortunately, MAGMAS/PAM16/TIM16 was not detected in our dataset, so we cannot answer the question, whether it was part of the 1.3 MDa complex.

Referee #2:
The authors have examined the mitochondrial complexome in cells lacking mtDNA (Rho zero cells) and in the parent cell line from which they were derived (143B osteosarcoma). They identified nearly 1000 mitochondrial proteins in this analysis, which represents a substantial proportion of the mitochondrial proteome in the Mitocarta database. This group is expert at complexome analysis and the data presented here are of high quality. The paper is entirely descriptive and therefore is more appropriately considered a resource for the mitochondrial community. There are, however, some rather unexpected observations, which could inform future functional studies. I have no concerns about the data quality or the presentation.

We thank the reviewer for commending the quality of our work. Indeed, this study was intended to mainly showcase the enormous potential of a comprehensive analysis by complexome profiling and to provide a valuable resource for further studies to the mitochondrial community. We have now also highlighted the resource character of our manuscript in the Abstract (line 32) and at the beginning of the Discussion (line 402).

Referee #3:
The paper by Guerrero-Castillo et al focuses in the analysis of the mitochondria complexes in rho0 cells. The main method is complexome profiling, which is the most appropriate method, demonstrated by several groups and studies, to analyze global changes in stress conditions and certain mutations in mitochondria. The method in itself is interesting because it provides insight and a large amount of useful information about the reorganization of the mitochondrial proteome in human rho0 cells and on what proteins/complexes/subcomplexes are stable in the absence of the mtDNA-encoded elements and the genome itself. Although I think this paper can be used as a useful resource for future studies, it lacks mainly in functional characterization of the changes observed and any mechanistic insight. It might be more appropriate to be published as a Resource paper in journals that are more appropriate for this type of work.
We thank the reviewer for carefully assessing our work and for highlighting the usefulness of our methodological approach and the information obtained in this way. Indeed, this study was intended to mainly showcase the enormous potential of a comprehensive analysis by complexome profiling and to provide a valuable resource for further studies to the mitochondrial community. We have now also highlighted the resource character of our manuscript in the Abstract (line 31) and at the beginning of the Discussion (line 402).

Major points:

- The paper is fundamentally descriptive and does not provide any clue about what on the functional implications of these reorganizations. Some key observations (for example lack of effects on MICOS subunits) are puzzling, and yet no effort has been made to address these from a mechanistic point of view. More general point which a non-specialist would wonder: why do rho0 cells need mitochondria at all? Some discussion on this in view of the data would be needed.

We agree with the reviewer that many of the observations highlighted in our manuscript call for follow up studies to address them mechanistically. However, given the enormous amount of information already obtained by our “horizontal” approach, we decided to stick with compiling the information that could be derived directly from our data and abstained from speculating about possible mechanistic implications. Addressing such questions experimentally would have been beyond the scope of this study. The question, why cells need mitochondria at all, if they can survive without mtDNA, cannot be answered in a straightforward way. Obviously, and as also illustrated by Figure 8, even without the OXPHOS system, mitochondria are a major metabolic hub and take part in many biosynthetic and catabolic pathways. Roland Lill and co-workers have shown that in yeast, mitochondria are essential because of their role in the synthesis of iron-sulfur clusters also needed for non-mitochondrial proteins. OXPHOS itself seems to be essential for de novo pyrimidine synthesis (lines 59-61). While the reviewer has a good point in saying that in the light of our results, non-specialists may wonder about the importance of mitochondria, these examples illustrate that answering this question appropriately cannot be done in a few sentences and would be better placed in a review article on rho0 cells. Overall, we felt that getting into this ultimately somewhat philosophical discussion would have distracted from the focus of our study showcasing the impressive plasticity of the mitochondrial complexome.

- The quantitative data are based on label-free peptide intensity quantifications, which depend on many technical variables. In fact, there seems to be a lot of variability among the four replicates both in rho0 and WT cells. It might be convenient to validate these quantifications using an independent method, for example WB and immunodetection of representative proteins.

The identification and label-free quantification of proteins by peptide-based LC/ESI-mass spectrometry is now a very well established and widely used approach that is at least as reliable both qualitatively and quantitatively as Western blotting and other approaches employing immunodetection. We would like to emphasize that by performing complexome profiling analysis of four biological replicates for each cell line, we compiled raw data from a total of 480 individual samples run on the mass spectrometer. This provided a level of data power, comprehensiveness and detail that is simply unachievable by Western-blotting. Inherently validating our quantitative analysis, many of the observed changes showed very high statistical significance and importantly were consistent with the changes in the averaged migration profiles. Overall, we do not think that performing Western blots for a few selected proteins and analyzing them densitometrically could further validate our results. Rather, we think that the documented variability among the four
replicates reflects typical differences between independent biological samples and as such may contain useful information in itself.

On page 4 when talking about the DNA and RNA polymerases etc, the solubilization and separation methods used for BNGE most probably eliminate the nucleic acids from the samples, so it is difficult to say how much of these factors are actually bound to DNA and RNA in the WT cells.

We thank the reviewer for highlighting this point. For the mitoribosomes we can be sure that the RNA was not degraded, since their subunits migrated at the apparent masses predicted including the rRNAs. To make this clear we have added the following sentence (line 98-99): “It can be concluded from these apparent masses that the two subunits of the mitochondrial ribosome were stable and retained their rRNAs under the experimental conditions used.” As for the other nucleic acid-binding protein complexes, we answered this question already in the original version of our manuscript concluding that the identical masses in control and rho0 cells showed that they did not contain nucleic acids (lines 128-129 and lines 140-141).

There are some points that should be discussed in the context of previous observations:

We are certainly aware of the enormous body of literature from several decades of research by numerous groups reporting on subcomplexes and assembly intermediates of OXPHOS complexes. To keep our study concise and focused, we had made the deliberate decision to keep referrals to previous work to the minimum necessary. However, we have gone a little too far in restraining ourselves and are grateful to the reviewer for the suggestions in this respect. We have considered all of them and addressed them as detailed below.

- In page 4, Oxidative phosphorylation section, the fact that there is an F1 subcomplex of complex V in the rho0 cells has been known for quite some time now. In fact, its presence was deemed necessary to maintain the membrane potential through ATP hydrolysis (Buchet, K., and Godinot, C. (1998). Functional F1-ATPase essential in maintaining growth and membrane potential of human mitochondrial DNA-depleted rho degrees cells. J Biol Chem 273, 22983-22989.) (Appleby, R.D., Porteous, W.K., Hughes, G., James, A.M., Shannon, D., Wei, Y.H., and Murphy, M.P. (1999). Quantitation and origin of the mitochondrial membrane potential in human cells lacking mitochondrial DNA. Eur J Biochem 262, 108-116.). This is not really compatible with the explanation the authors give for the reason why IF1 is found bound to this species in the rho0 cells, please discuss. Also, IF1 seems to be bound to the intermediates of CV during their assembly before the incorporation of the mtDNA-encoded subunits (He, J., Ford, H.C., Carroll, J., Douglas, C., Gonzales, E., Ding, S., Fearnley, I.M., and Walker, J.E. (2018). Assembly of the membrane domain of ATP synthase in human mitochondria. Proceedings of the National Academy of Sciences of the United States of America 115, 2988-2993.)

Indeed, the earlier observation that the F1 subcomplex helps to maintain the mitochondrial membrane potential and IF1 binding seems contradictory. Certainly, ATP hydrolysis needs to be controlled also in rho0 cells. We cannot address this unresolved issue based on our data, but have now referenced previous work as suggested (lines 153-160): “Both subassemblies of complex V have been observed previously in human rho cells and mtDNA depletion syndrome (Buchet & Godinot, 1998; Carrozzo et al, 2006). In controls, the ATP synthase inhibitor protein IF1 was mostly detected in free form, while it was almost completely bound to F1 and F1-c subcomplexes in rho cells (Figure 4B) as reported earlier (Carrozzo et al., 2006) and in line with a recent study on complex V assembly (He et al, 2018). Binding of the IF1 to F1 and F1-c subcomplexes could prevent wasteful ATP hydrolysis, although this reaction has
been shown to be used to maintain the membrane potential in \( \rho^0 \) mitochondria in conjunction with the nucleotide carrier ANT (Appleby et al, 1999; Buchet & Godinot, 1998).

- **Page 4-5**, when talking about the subcomplexes of complex III subunits, the accumulation of CYC1 together with UQCR10 was described in the MT-CYB mutated cells (Protasoni, M., Perez-Perez, R., Lobo-Jarne, T., Harbour, M.E., Ding, S., Penas, A., Diaz, F., Moraes, C.T., Fearnley, I.M., Zeviani, M., et al. (2020). Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV. The EMBO journal 39, e102817.) (Palenikova, P., Harbour, M.E., Prodi, F., Minczuk, M., Zeviani, M., Ghelli, A., and Fernandez-Vizarra, E. (2021). Duplexing complexome profiling with SILAC to study human respiratory chainassembly defects. Biochim Biophys Acta Bioenerg 1862, 148395.)

In fact, the cytochrome \( \text{c}_1 \)-subcomplex was described and biochemically characterized already a long time ago by Link et al. (1986). We have added a reference to this work as well as one of the recent papers suggested by the reviewer as follows (lines 168-171): “This subcomplex consisting of cytochrome \( \text{c}_1 \) and subunits UQCR10 and UQCRH was described and biochemically characterized already a long time ago (Link et al, 1986) and indications for this subassembly have been found recently also in cytochrome b deficient cells (Palenikova et al, 2021).”

- **Page 5**, when talking about complex IV subassemblies, the accumulation of COX4-COX5A subcomplexes was observed also in human cells defective in MT-CO1 and MTCO2 (Lobo-Jarne, T., Perez-Perez, R., Fontanesi, F., Timon-Gomez, A., Wittig, I., Penas, A., Serrano-Lorenzo, P., Garcia-Consuegra, I., Arenas, J., Martin, M.A., et al. (2020). Multiple pathways coordinate assembly of human mitochondrial complex IV and stabilization of respiratory supercomplexes. The EMBO journal 39, e103912.)

We have added this reference as suggested in line 175-176: “For complex IV, subassemblies containing subunits IV-1 and Va, not present in control cells but observed previously in cells deficient in mtDNA encoded complex IV subunits (Lobo-Jarne et al, 2020), accumulated in \( \rho^0 \) cells (Figure EV3H).”

- **Page 5**, when talking about complex II subassemblies, interestingly, the accumulation of SDHAF2 and SDHA subassemblies was also observed in MT-CYB mutants (Protasoni, M., Perez-Perez, R., Lobo-Jarne, T., Harbour, M.E., Ding, S., Penas, A., Diaz, F., Moraes, C.T., Fearnley, I.M., Zeviani, M., et al. (2020). Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV. The EMBO journal 39, e102817; Palenikova, P., Harbour, M.E., Prodi, F., Minczuk, M., Zeviani, M., Ghelli, A., and Fernandez-Vizarra, E. (2021). Duplexing complexome profiling with SILAC to study human respiratory chain assembly defects. Biochim Biophys Acta Bioenerg 1862, 148395.)

Although SDHAF2 accumulation has been reported in these studies, this subassembly is not explicitly discussed. The association of SDHAF2 with SDHA was first reported by Hao et al. (2009) and we have now included a reference to this work in line 185: “…because the fraction not bound to complex II was retained in a complex with the flavinylation assembly factor SDHAF2 described earlier (Hao et al, 2009), which was increased almost four-fold in abundance (Figure 4C).”

- **Page 6**, the increase in ANT2 levels in the \( \rho^0 \) would be compatible with its role in ATP transport in order to keep cell viability and mitochondrial membrane potential through ATP
While this seems like a possibility, it is a speculation to link the upregulation of ANT2 to the maintenance of the membrane potential by nucleotide transport in rho0 cells. Therefore, rather than citing the work by Buchet and Godinot again here, we referred to the link between ANT and preservation of the membrane potential in rho0 cells, when discussing the persistence of the F\textsubscript{1} subcomplex (see above and line 159).

In page 9, it is really striking that there are no changes in the MICOS complexes in the rho0 cells. How could the distribution of these complexes in the altered cristae of the rho0 cells be investigated further?

Indeed, the absence of any changes in the migration profiles of the components of the MICOS complex is one of the most striking observations of our study calling for further analysis. However, as explained in detail above, we decided to stick with the resource character of this study and do not feel that it is our task to provide instructions for possible follow-up studies.

Minor points:

- It would be good to have a graphical representation in Figure 8 of what OXPHOS subcomplexes accumulate in the rho0 cells.

We thank the reviewer for this suggestion. Taking advantage of the novel tool provided by The EMBO Journal to readily access further illustrations, we have prepared a new Expanded View figure (EV2) showing the submodules of the five OXPHOS complexes retained in rho0 cells.

- In page 10, last paragraph, it should be "OMA1" instead of "OMA2".

Thanks, this has been corrected.

- The authors mention during the results and discussion sections the fact that their datasets contain information about cytosolic components. However, how reliable can the quantifications be if there are differences in the presence of these 'contaminants' in the different mitochondrial fraction preparations used in the analysis. I think this is not a very well documented point and should not be part of this paper as it dilutes the message and is open to many interpretations.

Following the reviewer’s suggestion, we have removed the analysis of the cytosolic components and all references to it from the manuscript.
23rd Aug 2021

Re: EMBOJ-2021-108648R
Ablation of mitochondrial DNA results in widespread remodeling of the mitochondrial complexome

Dear Prof. Brandt,

Thank you for submitting your revised manuscript. Please excuse the delay in communicating this decision to you, which was due to delayed referee responses over the summer holiday period, as well as absences from the office. We have now received the reports from the two initial referees (please see comments below) and I am pleased to say that they overall find that their comments have been satisfactorily addressed and now support publication. Therefore, I would now ask you to address the two editorial points that are listed in detail below. Once these remaining issues are resolved, we will be happy to formally accept the manuscript for publication.

I look forward to receiving your final revision. Please feel free to contact me if you have further questions.

Kind regards,

Stefanie Boehm
Editor
The EMBO Journal

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Referee #1:
The authors addressed my concerns in full in their revised version of the manuscript. The presented study is of excellent quality and provides a rich source on alterations of the proteome and complexome in rho0 cells that are highly interesting for the broad readership in cell biology. I strongly recommend this manuscript for publication in EMBO J.

Referee #3:
The authors provided a thoroughly revised version of their ms and have taken into consideration the points raised in the first review round. I still think this paper should be published as a Resource paper and indicated as such, which will in fact may attract the broader interest of researchers from outside the field. I am happy to recommend publication of this revised version.

Stefanie Boehm
Editor
The EMBO Journal
Referee #1:

The authors addressed my concerns in full in their revised version of the manuscript. The presented study is of excellent quality and provides a rich source on alterations of the proteome and complexome in rho0 cells that are highly interesting for the broad readership in cell biology. I strongly recommend this manuscript for publication in EMBO J.

Referee #3:

The authors provided a thoroughly revised version of their ms and have taken into consideration the points raised in the first review round. I still think this paper should be published as a Resource paper and indicated as such, which will in fact may attract the broader interest of researchers from outside the field. I am happy to recommend publication of this revised version.
The authors performed the requested editorial changes.
Thank you again for submitting the final revised version of your manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.
EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: [Insert name]
Journal Submitted to: [Insert journal name]
Manuscript Number: [Insert manuscript number]

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal’s authorship guidelines in preparing your manuscript.

A. Figures

1. Data

The data shown in figures should satisfy the following conditions:

- The data were obtained and processed according to the field’s best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- Figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- Graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- If n is 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified.
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- A specification of the experimental system investigated (eg cell line, species name).
- The assay(s) and method(s) used to carry out the reported observations and measurements.
- An explicit mention of the biological and chemical entity(ies) that are being measured.
- An explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- The exact sample size (n) for each experimental group/condition, given as a number, not a range.
- A statement of how many times the experiment shown was independently replicated in the laboratory.
- Definitions of statistical methods and measures:
  - Common tests, such as t-tests (please specify whether paired vs. unpaired), Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section.
  - Are tests one-sided or two-sided?
  - Are there adjustments for multiple comparisons?
  - Exact statistical test results, e.g., P values = x but not P values < x; yes, based on simple Student’s t-test analysis
  - Are there adjustments for multiple comparisons?
  - Definition of ‘center values’ as median or average;
  - Definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself.

Every question should be answered. If the question is not relevant to your research, please write NA (non-applicable).

We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B. Statistics and general methods

| Question                                                                 | Yes | No | NA | Comment |
|-------------------------------------------------------------------------|-----|----|----|---------|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? |     |    |    | Standard sample size for a biochemical/proteomics experiment (n=4) |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | n.a. |    |    |         |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | n.a. |    |    |         |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g., randomization procedure)? If yes, please describe. | n.a. |    |    |         |
| For animal studies, include a statement about randomization even if no randomization was used. | n.a. |    |    |         |
| 4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and assessing results (e.g., blinded of the investigator)? If yes please describe. | n.a. |    |    |         |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | n.a. |    |    |         |
| 5. For every figure, are statistical tests justified as appropriate? | yes |    |    |         |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | yes, based on simple Student’s t-test analysis |    |    |         |
| In there an estimate of variation within each group of data? | n.a. |    |    |         |

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- http://www.consort-statement.org/checklists/view/32-consort/66-title
- http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumour-marker-prognostic-studies-remark/
- http://www.ncbi.nlm.nih.gov/pmc/
C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile, e.g., Antibodypedia (see link list at top right), IDGewebe (see link list at top right).

7. Identify the sources of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.

9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.

10. We recommend consulting the ARRIVE guidelines (see link list at top right) (Fucci Bid. 5(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under ‘Reporting Guidelines’. See also: NIH (see link list at top right) and NRC (see link list at top right) recommendations. Please confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.

12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

13. For publication of patient photos, include a statement confirming that consent to publish was obtained.

14. Report any restrictions on the availability (and/or on the use) of human data or samples.

15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.

16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under ‘Reporting Guidelines’. Please confirm you have submitted this list.

17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under ‘Reporting Guidelines’. Please confirm you have followed these guidelines.

F- Data Accessibility

18: Provide a “Data Availability” section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RINSeq data: Gene Expression Omnibus GSE19462, Proteomics data: PRIDE P2603283 etc.). Please refer to our author guidelines for ‘Data Deposition’.

Data deposition in a public repository is mandatory for:

- a. Protein, DNA and RNA sequences
- b. Macromolecular structures
- c. Crystallographic data for small molecules
- d. Functional genomics data
- e. Proteomics and molecular interactions

Deposition is strongly recommended for any datasets that are central and integral to the study, please consider the journal’s data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under ‘Expanded View’ or in unstructured repositories such as Citrino (see link list at top right) or Dryad (see link list at top right)).

19. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or ECB (see link list at top right).

20. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized formats (e.g. CslML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (e.g. CslML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM

G- Dual use research of concern

21. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/USDA) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.