EMBO reports

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Structural coupling of the EF hand and C-terminal GTPase domains in the mitochondrial protein Miro

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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 05 June 2013

Thank you very much for the submission of your research manuscript to our editorial office and your patience while we were conducting the peer review.

As the detailed reports are pasted below I will only summarize the main points here. You will see that all reviewers appreciate the interest of your findings and support publication of your study in our journal. However, they also point out aspects of your study that would need to be further discussed and/or clarified. These are for the most part minor issues, but I would kindly ask you to address them when submitting the revised version. Referee 2 states that the resolution of some of the structures could be increased (comment #1) and if you are able to do so -or have already done so in the meantime- I would encourage you to include this data. This would, however, not be a prerequisite for publication.
Given these positive evaluations, the reviewers constructive comments and the potential interest of the study, 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.

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). If you feel that the additional data and discussions requested by the reviewers would make the manuscript too long you may consider including some peripheral data in the form of Supplementary information. However, materials and methods essential for the repetition of the key experiments should be described in the main body of the text and may not be displayed as supplemental information only.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

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:

This is a very interesting study on the crystal structure of the EF-hands and the second GTPase domain of the GTPase Miro. The resolved structure demonstrates some very interesting and unexpected features of Drosophila Miro that will have a significant impact on our understanding of the mechanisms underlying the regulatory function of the Miro GTPases.
Specific comments:
1. The structure describes the Drosophila Miro and it is mentioned in the text, but only if you read carefully. I think it would be a good idea to mention already in the abstract that this is the Drosophila protein.
2. The authors mention in the abstract "Phosphorylation of Miro by Pink1 kinase and its subsequent Parkin-mediated degradation leads to mitophagy of damaged mitochondria". In addition, the authors speculate about the structural implication of phosphorylation of S324. It is actually not clear if phosphorylation is regulating the stability of Miro. This was an attractive model put forward in reference 12. However, it involved another serine residue, S156 (S182 IN dMiro). Moreover, Liu et al. (reference 13) did not detect any PINK1-dependent phosphorylation of Miro. Thus, Miro phosphorylation is still an open, yet attractive, hypothesis. I suggest that the authors tone this down.
3. The cGTPase domain is apparently able to bind MgGDP but can it also bind GTP? Is there any hint of a conformational switch? The apo-, Ca- and MgGDP-conformations cannot be expected to be so much different in this regard.
4. The authors state that the nucleotide-binding pocket in the Apo- and MgGDP-bound states is unusually open, what does it mean?

Referee #2:

In this manuscript, Klosowiak and colleagues report the crystal structure of residues 201-617 of Drosophila Miro that encompasses the EF hands and C-terminal GTPase domain. The work is important since structures of many key components of the mitochondrial morphology machinery are lacking and they can contribute insights at the molecular level. Furthermore, the structure of Miro is topical given the very recent findings by Nunnari and colleagues on the function of the Miro yeast ortholog Gem1 in linking ERMES with mitochondrial fission (published in eLife, which should be cited). The structure reveals the presence of an additional hidden EF hand with each canonical EF hand and a helix that acts as a ligand mimetic (making a newly-coined "ELM" domain). While the C-terminal GTPase domain makes extensive contact with one EF hand, the authors find that the structure is not significantly altered by ion or nucleotide binding, thus pointing to additional mechanisms of regulation. The manuscript is well written and the work has not been over interpreted.
Main comments:
1.The authors seemed to have been very conservative when deciding on how far the data go in terms of resolution. They may have been able to push the data further, and they are likely to be throwing away valuable high resolution data. Particularly the Mg-GDP complex has 7 sigma in the highest resolution shell, and they stopped it at 3.1 A resolution.

2.The source of Ramachandran outliers in the structures should be clarified - one should work hard to fix these.

3.The authors have not performed any site directed mutagenesis on Miro1/Gem1 to determine the importance of key residues with respect to function. I can understand that this would make it a bigger paper and so we can expect other groups to do this as a result of the publication. They have however made some mention of key residues that have been previously shown to be important/modified. I would have liked to see a little more interpretation of this aspect with some of the relevant supplemental figures being brought into the main body of the manuscript so that they aren't lost (e.g. Supp Fig 5 incorporated as a panel in Fig. 4).

4.I think it is interesting that the calcium/nucleotide binding does not impart much change in the structure. Perhaps the authors need to comment on whether the addition of the other GTPase domain might act here (I doubt it will but it's worth commenting on this limitation of the work).

5.The authors have been selective about their raw SAXS data, and only show some of it for one of the structures, but not the rest. I think it would be good to include the I(q) versus q plots for all samples described in the paper.

6.The following sentence on page 3 sound a little too melodramatic: "Two linker regions, which follow a tortuous path across the "bottom" of the molecule, join the three domains." Consider rewording.

Referee #3:

Miro is a membrane-anchored protein of the mitochondrial outer membrane and is similar to Rho-like GTPases. In addition to two GTPase domains Miro has 2 EF hands. The protein Milton mediates the Ca-dependent contact of mitochondria via Miro to a kinesin which can move mitochondria through the cell along microtubules. This is especially important in neuronal cells where a nonfunctional Miro leads to neuronal dysfunction. Miro is one of the targets of PINK1 and Parkin leading to degradation of the protein together with the potentially damaged mitochondrion that Miro is attached to. Miro is also located in mitochondria-ER contact sites and may play additional roles, e.g., in Ca signaling between ER and mitochondria. Here, the authors present several crystal structures of a central fragment of Miro lacking its N-terminal GTPase domain and its C-terminal anchor. While it is normal that the membrane anchors are not included in crystallization attempts, the omission of the important N-terminal piece is a severe shortcoming for the functional conclusions one might want to make from this study. Nevertheless, the study is well performed and even this crystallized fragment may be helpful for future functional studies. The study shows that the two EF hands are arranged in an unusual, so far undescribed fashion. The two "ELM" domains are similar but show characteristic differences that may be a common feature of

Miro proteins from other species. A unique feature is the interaction (over a large contact area) of an EF hand and the cGTPase domain. One may speculate that the N-terminal GTPase domain interacts in a similar fashion with EF hand 1. Another interesting feature is the location of a PINK1 phosphorylation site at the interface of ELM2 and the cGTPase domain. The authors use this to speculate about the functional consequences of phosphorylation, but no direct studies addressing this issue were performed here. There is also no real clue from the current structures as to how Ca binding to Miro (its EF hands) might interfere with mitochondrial movement along the microtubules. Thus, more work is needed to really understand Miro's important cellular function. The current manuscript is a good start for dedicated future mutational and functional studies. Since it can stimulate research in this area it may be suitable for publication.

Concerns:
1. The title is misleading. The structure reported is not the complete structure of the mitochondrial outer membrane protein Miro, but only a fragment excluding, e.g., the membrane part. This should be clear from the title.
2. The last part of the last sentence of Abstract should better read "... and phosphorylation-dependent regulation of mitochondrial function by Miro."
3. Supplementary table 1 of crystallographic statistics shows datasets from 2.61-3.10 A, while in the abstract the authors say 2.79-3.10 A. Please, homogenize.
4. The rationale for modeling the electron density as homoserine is not clear. The size and shape of the density may suggest many other molecules. Unless there is a functional relevance to this density being a homoserine molecule, my suggestion would be to calculate and include an unbiased experimental electron density map, 'Omit map' as a supplementary figure.
5. Methods: Replace "dripping" by dipping".
6. The wavelength of data collection should be included in the supplementary table 1. Especially if the data for the SeMet was collected at the 'Se' edge.
7. The text for supplementary Figure 1 suggests that a calcium ion was present. If so, it should be included as a ligand in the suppl. Table 1.
8. The Rfree value of 0.4552 for the last shell seems high; also in comparison to the other datasets reported here.
9. Some of the suppl. References are incomplete.

1st Revision - authors' response 24 July 2013

Detailed replies to reviewer critiques of MS# EMBOR-2013-37508-T:

Referee #1:

This is a very interesting study on the crystal structure of the EF-hands and the second GTPase domain of the GTPase Miro. The resolved structure demonstrates some very interesting and unexpected features of Drosophila Miro that will have a significant impact on our understanding of the mechanisms underlying the regulatory function of the Miro GTPases.
Specific comments:
1. The structure describes the Drosophila Miro and it is mentioned in the text, but only if you read carefully. I think it would be a good idea to mention already in the abstract that this is the Drosophila protein.

We have reworded the abstract to read:

"Here we present crystal structures comprising the tandem EF hand and C-terminal GTPase (cGTPase) domains of ***Drosophila*** Miro."

2. The authors mention in the abstract "Phosphorylation of Miro by Pink1 kinase and its subsequent Parkin-mediated degradation leads to mitophagy of damaged mitochondria". In addition, the authors speculate about the structural implication of phosphorylation of S324. It is actually not clear if phosphorylation is regulating the stability of Miro. This was an attractive model put forward in

reference 12. However, it involved another serine residue, S156 (S182 IN dMiro). Moreover, Liu et al. (reference 13) did not detect any PINK1-dependent phosphorylation of Miro. Thus, Miro phosphorylation is still an open, yet attractive, hypothesis. I suggest that the authors tone this down.

We agree that Miro phosphorylation by Pink1 as part of a Pink1/Parkin mitochondrial quality control pathway is an attractive model, and we recognize that there is conflicting evidence in the literature. The sentence quoted above has been removed from the abstract. We have devoted some discussion to the issue in the newly written introduction, with this sentence:

"While it is clear that Pink1 is a necessary component in this Miro-Pink1-Parkin pathway, whether direct phosphorylation of Miro is required to trigger Parkin-mediated degradation is contested [12,13]."

We have also toned down our speculation with the following sentence changes in the Results and Discussion section:

"Both nucleotide binding and Pink1 phosphorylation could potentially modulate the structure of Miro **within** the ELM2/cGTPase interface."

"**One of several potential** Pink1 phosphorylation site lies at the heart of the network of polar contacts in the third region of the ELM2/cGTPase interface."

"Pink1 phosphorylation **has been shown to trigger** Parkin-dependent degradation of Miro. A very recent report [22] has identified several Parkin ubiquitination sites in human Miro, four of which map onto our structure on the same face as the **S324** Pink1 site **(Fig 5)**."

3. The cGTPase domain is apparently able to bind MgGDP but can it also bind GTP? Is there any hint of a conformational switch? The apo-, Ca- and MgGDP-conformations cannot be expected to be so much different in this regard.

Whether the cGTPase of *Drosophila* Miro specifically binds GTP has not been formally tested, and the functional significance of GTP binding is unclear. We have expanded our discussion of potential GTP binding to the cGTPase domain:

"The Switch I threonine typically serves a γ-phosphate-sensing role in GTPases [17]. **Although dMiro T481 is not evolutionarily conserved, a γ-phosphate-sensing mechanism enabling the Miro Switch I loop to access the nucleotide-binding pocket upon MgGTP-binding** would alter the first region of the ELM2/cGTPase interface (Fig 4A, B)."

4. The authors state that the nucleotide-binding pocket in the Apo- and MgGDP-bound states is unusually open, what does it mean?

We understand that the terminology "unusually open" is rather ambiguous and have reworded the sentence to read:

"An extensive interface between hEF2 and the cGTPase involves the nucleotide-responsive Switch I element, which is **positioned away from the nucleotide-binding pocket** in both the apo and MgGDP-bound structures (supplementary Fig S1 online)."

Referee #2:

In this manuscript, Klosowiak and colleagues report the crystal structure of residues 201-617 of Drosophila Miro that encompasses the EF hands and C-terminal GTPase domain. The work is important since structures of many key components of the mitochondrial morphology machinery are

lacking and they can contribute insights at the molecular level. Furthermore, the structure of Miro is topical given the very recent findings by Nunnari and colleagues on the function of the Miro yeast ortholog Gem1 in linking ERMES with mitochondrial fission (published in eLife, which should be cited).

This reference has been added as **reference number 10**.

The structure reveals the presence of an additional hidden EF hand with each canonical EF hand and a helix that acts as a ligand mimetic (making a newly-coined "ELM" domain). While the C-terminal GTPase domain makes extensive contact with one EF hand, the authors find that the structure is not significantly altered by ion or nucleotide binding, thus pointing to additional mechanisms of regulation. The
manuscript is well written and the work has not been over interpreted.
Main comments:
1.The authors seemed to have been very conservative when deciding on how far the data go in terms of resolution. They may have been able to push the data further, and they are likely to be throwing away valuable high resolution data. Particularly the Mg-GDP complex has 7 sigma in the highest resolution shell, and they stopped it at 3.1 A resolution.

We reprocessed the X-ray data to slightly higher resolution. The resulting changes were very minor and did not affect our results or their interpretation. Supplementary Table 1 now shows inclusion of these data, and we now briefly note in the Supplementary Methods that diffraction anisotropy limits the achievable resolution.

2.The source of Ramachandran outliers in the structures should be clarified - one should work hard to fix these.

These have been corrected.

3.The authors have not performed any site directed mutagenesis on Miro1/Gem1 to determine the importance of key residues with respect to function. I can understand that this would make it a bigger paper and so we can expect other groups to do this as a result of the publication. They have however made some mention of key residues that have been previously shown to be important/modified. I would have liked to see a little more interpretation of this aspect with some of the relevant supplemental figures being brought into the main body of the manuscript so that they aren't lost (e.g. Supp Fig 5 incorporated as a panel in Fig. 4).

We agree that incorporating Supplementary Figure S5 into the main body would strengthen the paper by bringing these sites of posttranslational modification to the reader's attention in the context of this new structure. **Therefore, we have moved Supplementary Figure S5 to a newly created Figure 5.**

In keeping with this reviewers' praise for not over-interpreting our work, we feel that we have adequately addressed the important/modified key residues visible in the structure in the text of the paper and should avoid further speculation, especially given the controversy in the literature regarding the mechanism of Ca^{2+}-mediated mitochondrial arrest as well as Miro phosphorylation by Pink1, now mentioned in the introduction.

4.I think it is interesting that the calcium/nucleotide binding does not impart much change in the structure. Perhaps the authors need to comment on whether the addition of the other GTPase domain might act here (I doubt it will but it's worth commenting on this limitation of the work).

In Supplementary Figure S9, we show SEC-MALS data for both MiroS and the full-length MiroL (which includes the nGTPase but lacks the TM domain) +/- Ca^{2+}. It indicates that MiroL does not change its oligomeric state in response to Ca^{2+}. Importantly, the respective SEC-MALS elution

profiles for both MiroS and MiroL +/- Ca^{2+} are superimposable, suggesting that the presence of the nGTPase does not greatly alter the behavior of Miro in this assay.

Because we do not present high-resolution crystallographic or SAXS data of the MiroL protein, we would rather not speculate further on its structure. That said, the reviewer makes an excellent point. It is entirely plausible that the nGTPase domain or the TM region contribute a key structural role to calcium/nucleotide binding response that cannot be appreciated in the MiroS construct/structure.

We now discuss this issue a bit further in presenting the SEC-MALS data:

"SEC-MALS shows that MiroS, and a longer Miro construct containing the nGTPase domain (aa 1-617, referred to as MiroL for "long"), are both monomers in solution regardless of Ca^{2+} concentration (supplementary Fig S9 online), consistent with the SAXS data for MiroS. **Additionally, the respective elution profiles for both MiroS and MiroL ± Ca^{2+} are superimposable. A dramatic conformational rearrangement in response to Ca^{2+} might be expected to alter the shape of the protein and slightly change its elution profile. The fact that we observe no change suggests that the presence of the nGTPase does not greatly alter the behavior of Miro in this assay.**"

5.The authors have been selective about their raw SAXS data, and only show some of it for one of the structures, but not the rest. I think it would be good to include the I(q) versus q plots for all samples described in the paper.

We have created a new Supplementary Figure S8, which shows the scattering curves and Guinier plots for the non-Ca conditions, in response to this suggestion. In addition, we expanded Supplementary Figure S7 to show scattering curves, Guinier, P(r), and Kratky plots for each of the three reconstructions (Miro-Ca, Miro-Ca+MgGTP, and Miro-Ca+MgGDP) for completeness.

The notion that the SAXS data corroborate the crystal structure as stated in our original manuscript is correct, but rather superficial. We therefore changed this statement to include a more detailed description of our SAXS data:

"Radii of gyration (Rg) and *ab initio* molecular envelope reconstructions from SAXS experiments indicate that the overall structural shape of MiroS bound to Ca^{2+} in solution corroborates the crystallographic data. The MiroS Rg under apo or Mg^{2+} conditions also remains similar to the crystal structures, indicating that the MiroS structure is not grossly altered by ion or nucleotide binding (supplementary Fig S7-8 and Table S2 online). The increased Dmax and Rg of apo-Miro may indicate that Miro is less stably folded without Ca^{2+}, like several other EF hand proteins [14,21]. Together, these data indicate that gross conformational rearrangements of MiroS into another stable form and/or oligomerization of MiroS in the conditions tested did not occur."

6.The following sentence on page 3 sound a little too melodramatic: "Two linker regions, which follow a tortuous path across the "bottom" of the molecule, join the three domains." Consider rewording.

This sentence now reads:

"Two linker regions, which follow a **circuitous** path across the "bottom" of the molecule, join the three domains."

Referee #3:

Miro is a membrane-anchored protein of the mitochondrial outer membrane and is similar to Rho-like GTPases. In addition to two GTPase domains Miro has 2 EF hands. The protein Milton mediates the Ca-dependent contact of mitochondria via Miro to a kinesin which can move mitochondria through the cell along microtubules. This is especially important in neuronal cells where a nonfunctional Miro leads to neuronal dysfunction. Miro is one of the targets of PINK1 and

Parkin leading to degradation of the protein together with the potentially damaged mitochondrion that Miro is attached to. Miro is also located in mitochondria-ER contact sites and may play additional roles, e.g., in Ca signaling between ER and mitochondria. Here, the authors present several crystal structures of a central fragment of Miro lacking its N-terminal GTPase domain and its C-terminal anchor. While it is normal that the membrane anchors are not included in crystallization attempts, the omission of the important
N-terminal piece is a severe shortcoming for the functional conclusions one might want to make from this study. Nevertheless, the study is well performed and even this crystallized fragment may be helpful for future functional studies. The study shows that the two EF hands are arranged in an unusual, so far undescribed fashion. The two "ELM" domains are similar but show characteristic differences that may be a common feature of Miro proteins from other species. A unique feature is the interaction (over a large contact area) of an EF hand and the cGTPase domain. One may speculate that the N-terminal GTPase domain interacts in a similar fashion with EF hand 1. Another interesting feature is the location of a PINK1 phosphorylation site at the interface of ELM2 and the cGTPase domain. The authors use this to speculate about the functional consequences of phosphorylation, but no direct studies addressing this issue were performed here. There is also no real clue from the current
structures as to how Ca binding to Miro (its EF hands) might interfere with mitochondrial movement along the microtubules. Thus, more work is needed to really understand Miro's important cellular function. The current manuscript is a good start for dedicated future mutational and functional studies. Since it can stimulate research in this area it may be suitable for publication.

Concerns:
1. The title is misleading. The structure reported is not the complete structure of the mitochondrial outer membrane protein Miro, but only a fragment excluding, e.g., the membrane part. This should be clear from the title.

The title has been changed to more explicitly describe the findings in the paper:

“Structural coupling of the EF hand and C-terminal GTPase domains in the mitochondrial protein Miro”.

2. The last part of the last sentence of Abstract should better read "... and phosphorylation-dependent regulation of mitochondrial function by Miro."

The change has been made.

3. Supplementary table 1 of crystallographic statistics shows datasets from 2.61-3.10 A, while in the abstract the authors say 2.79-3.10 A. Please, homogenize.

Resolution information has been removed from the abstract due to space constraints, and has been homogenized throughout the remainder of the text.

4. The rationale for modeling the electron density as homoserine is not clear. The size and shape of the density may suggest many other molecules. Unless there is a functional relevance to this density being a homoserine molecule, my suggestion would be to calculate and include an unbiased experimental electron density map, 'Omit map' as a supplementary figure.

We modeled in homoserine essentially as a “placeholder" following careful inspection of the density and a survey of the PDB ligand database. However, we agree that the size and the shape of the density may suggest other molecules, and it is now referred to as an “unidentified ligand” or “UNL” in both the text of the paper and the coordinate files. We now show an "omit" Fo-Fc difference electron density map, obtained following refinement of the structure without the ligand included, in the new Supplementary Figure S3.

The rationale for modeling the electron density as homoserine is explained briefly in the Methods:

“Based on the size and shape of the electron density and a survey of the PDB ligand database, we have modeled the feature as homoserine, but the resolution of the electron density maps is not

sufficient to unambiguously identify it. Its origin, identity, and even whether it represents the ordered fragment of a larger molecule, remain unknown."

5. Methods: Replace "dripping" by dipping".

This is not in fact a typo, since flash freezing of the resuspended bacterial cell paste was accomplished by dripping the thick paste into liquid nitrogen in a drop-wise manner. This sentence has been changed to clarify:

"…flash frozen by dripping **drop-wise** into liquid N_2 (LN_2), and stored at - 80 °C."

6. The wavelength of data collection should be included in the supplementary table 1. Especially if the data for the SeMet was collected at the 'Se' edge.

The data for the structures was in fact collected at the 'Se' edge, and the wavelength of data collection is listed in the Methods as 0.97872 Å.

7. The text for supplementary Figure 1 suggests that a calcium ion was present. If so, it should be included as a ligand in the suppl. Table 1.

Ligand information has been added to Supplementary Table S1.

8. The Rfree value of 0.4552 for the last shell seems high; also in comparison to the other datasets reported here.

This has been addressed, please see Supplementary Table S1.

9. Some of the suppl. References are incomplete.

The references have been corrected.

2nd Editorial Decision 05 August 2013

Thank you for your patience while we have reviewed your revised manuscript. As you will see from the reports below, the referees are now all positive about its publication in EMBO reports. I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once you have shortened it slightly so that it conforms to our format. I might have mentioned before that manuscript should be not longer than 30,000 characters, including references. Your manuscript is over 36,000 characters at the moment and and I would very much appreciate if you could shorten it so that it is roughly 30,000 characters. Please note that this should not be done by simply moving all materials and methods into the supplementary section, but I am sure you will find ways to shorten the text in other places as well.

Once you have done this, please simply send us the revised text by email and we will upload it into our system. You will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports. I am very much looking forward to seeing your study in print soon.

REFEREE REPORTS:

Referee #2:

The authors have adequately addressed the minor concerns raised and I believe it is now suitable for publication.

Referee #3:

The manuscript has been revised in a satisfactory fashion. I recommend its publication.

Concerns:
1. The title is much more informative now.
2. Resolved.
3. Resolved.
4. Resolved.
5. Resolved.
6. Resolved.
7. Resolved.
8. Resolved.
9. Resolved.

2nd Revision - authors' response 21 August 2013

We shortened the manuscript as you suggested. Some detailed material was moved to the supplemental material, so both the main document and the revised supplement are attached here.

The revised manuscript is 30,920 characters.

In addition, we added PDB ID numbers to the manuscript and supplement, as the structures have been deposited in the database.

Please let me know if you need anything else.

3rd Editorial Decision 22 August 2013

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

