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# Structural basis for nuclear export activity of Importin13

Marlene Grünwald, Daniela Lazzaretti and Fulvia Bono

*Corresponding author: Fulvia Bono, Max-Planck-Institute for Developmental Biology*

**Review timeline:**

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| Submission date: | 07 November 2012 |
| Editorial Decision: | 19 December 2012 |
| Revision received: | 14 January 2013 |
| Editorial Decision: | 16 January 2013 |
| Revision received: | 28 January 2013 |
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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 19 December 2012

Thank you for submitting your research manuscript (EMBOJ-2012-83857) to our editorial office. It has now been seen by three referees and their comments are provided below.

All reviewers appreciate your study and are in general supportive of publication in The EMBO Journal. Nevertheless, both referee #2 and #3 do express several points of criticisms that should be experimentally addressed based on the reviewer's constructive suggestions. Given the comments provided, I would like to invite you to submit a suitably revised manuscript to The EMBO Journal that attends to the raised concerns in full. I should add that it is our policy to allow only a single major round of revision and that it is therefore important to address the raised concerns at this stage. Please do not hesitate to contact me should any particular argument require further clarification.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

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REFEREE REPORTS

Referee #1

Imp13 is an unusual karyopherin that mediates both nuclear import and export. Previous structural studies showed how Imp13 recognizes import cargoes (Mago-Y14 and Ubc9) and how RanGTP competes with import cargoes. The Grunwald et al manuscript now describes the crystal structures of Imp13-RanGTP-eIF1A complex and unliganded Imp13. Unfortunately, the structure of the export complex was determined at low resolution (3.6 angstrom) and it can be questionable if reliable interpretations can be made at the residue and atomic level at this resolution. However, the model is supported by mutagenesis experiments and so provides a plausible model of how Imp13 and RanGTP cooperatively recognize the export cargo (eIF1A). Moreover, comparison with the previously determined import complexes provides structural explanation for how competition between import and export cargoes for the binding to Imp13 can be important for the bidirectional transport. Overall, this is a nice structure-function study with significant impact for the nuclear transport field.

Minor comments:

1. p. 8, the last paragraph, "very similar to the structure of RanGTP in isolation": To my knowledge, the structure of RanGTP in isolation has never been reported. I guess here the authors compare their structure to the structure of Ran in the binary RanGMPPNP-RanBD complex (Vetter et al., 1999b).

2. p. 21, typo: hortologue -> orthologue

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6. Figure 7: The legend is too short. This is an important summary figure of this study, and the significance of this study would be conveyed more effectively if the legend were more descriptive. Also, this figure may be misleading in that all of the nuclear and cytosolic states of Imp13 are depicted in exactly the same shape. Depicting the conformational changes of Imp13 associated with binding/dissociation of proteins may help improve this figure.

Referee #2

Grünwald et al. present two new structures of Importin13, a bidirectional nucleocytoplasmic transport factor. Importin13 mediates the import of the Mago:Y14 heterodimer, a part of the exon junction complex, and the E2 SUMO-conjugating enzyme Ubc9. In addition, Importin13 exports the initiation factor eIF1A. Structures of the Importin13:RanGTP, Importin13:Mago:Y14, and Importin13:Ubc9 complexes have previously been determined. The first new structure is a heterotrimeric export complex, composed of Importin13, RanGTP, and eIF1A, in which the cargo, eIF1A, makes contacts with both Importin13 and RanGTP, solved at 3.6 Å resolution. The authors also present a 3.0 Å structure of apo Importin13, which is in an open conformation as opposed to the closed conformation observed in the heterotrimeric export complex. Using their structure of the export complex, the authors identify residues critical for eIF1A interaction and use them to probe the consequences of disrupting the eIF1A interaction in vivo. Additionally, combined with the previously solved structures of two different import complexes, the authors suggest a model where the conformational state of Importin13 acts as a switch for its import/export activity.

Building on previous structures of Importin13 import complexes, the structure of the Importin13:eIF1A:RanGTP export complex makes Importin13 the first karyopherin with structures known for both import and export complexes. With structures of many key steps in the Importin13 transport cycle, the authors generate a plausible, general model of how bidirectional karyopherins can control transport directionality. In the case of Importin13, import cargos cannot bind to Importin13 in the presence of RanGTP either due to steric clashes or direct competition, while export cargos can bind only in the presence of RanGTP.

Overall, the manuscript is well written and the presented data represent an important advance in the nucleocytoplasmic transport field and is of considerable interest for the broad readership of the EMBO Journal. If the authors satisfactorily address the points below, the paper should be published without delay.

Major comments

1. The interaction between eIF1A and the Importin13:RanGTP complex is relatively weak. Could the authors address the strength of this interaction in the context of other export complexes?
2. Imp13 appears to be essential in vivo, but it is unclear if the viability defect can be attributed to any of the structurally characterized interactions. Do any of the mutations disrupting cargo binding affect viability?
3. Overall, the manuscript contains a lot of redundant information and it should be considered to shorten the manuscript and focus on the new information.

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1. Only one methionine sidechain is visible in Figure S2A. Were the other two sidechains modeled? If so, they should be included in the figure. There is also a misspelling of methionine in the figure legend.
2. It would be nice to have an additional supplemental figure to complement the discussion comparing the Imp13 export complex to Crm1.
3. The color/pattern choice for Figure 2D is not very easy to figure out. Could one of the green curves be given a pattern or could a different color scheme be used?
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6. Please add the following information to Table 1: Total number of observations, number of unique observations in the last shell, redundancy in the last shell, total number of reflections used in refinement, number of reflections in the test set, number of refined atoms, number of solvent or ligand atoms, average B factors of protein and solvent atoms, and MolProbity score. Please include an appropriate reference for MolProbity.
7. Figure 7: please include the NPC in the figure and label the cytoplasm and nucleus.

Referee #3

In this study, the authors focused on Importin 13 (Imp 13) to know how Imp 13 can mediate both import and export of cargoes as a dual functional protein. They solved the crystal structures of Imp 13 in complex with RanGTP and one known export cargo eIF1A and the unbound state. They found that Imp 13 adopts a compact conformation with RanGTP where eIF1A recognizes the inner surface of Imp 13. Furthermore, it was shown that contacts on Imp 13 and on RanGTP stabilize export cargo binding. On the other hand, it has been shown that the association of Imp 13 with RanGTP suppresses that with the import cargo, indicating that the import cargo is released in the nucleus. The unbound form of Imp 13 revealed an open conformation, explaining export cargo release in the cytoplasm. In addition, they demonstrated that overexpression of Imp 13 induced cytoplasmic accumulation of eIF1A, meaning that Imp 13 exports eIF1A in vivo. They also observed that siRNA specific for Imp 13 showed a significant decrease in cell viability, indicating that the function of Imp 13 is essential. Taken together, the authors propose that the combination of shape, charge and size of the bound cargo drives the concomitant or exclusive interactions with RanGTP and determines the directionality of the transport and the delivery of the cargoes in the appropriate compartment. Thus, the data in this study are convincing and interesting, and this study provides the structural basis to understand the dual functions of Imp 13. The following point should be addressed before publication.

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In order to confirm the in vivo roles of Imp 13 on the import and export of proteins, the authors should examine the subcellular localization of endogenous Mago-Y14 and eIF1A proteins in the Imp 13-knockdown cells.

1st Revision - authors' response 14 January 2013

Referee #1

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*Thank you for pointing this out. We now changed the sentence into: "very similar to the structure of RanGTP in complex with RanBD1"*

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*Corrected*

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*We modified to: "prior to setting up crystallization trials"*

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*We now changed both the color and the pattern of the traces for better visibility.*

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*Following the reviewer's suggestion, we expanded the figure legend and modified the schematics of Imp13 states to convey an impression for the different conformational changes occurring in the cycle.*

Referee #2

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Major comments

1. The interaction between eIF1A and the Importin13:RanGTP complex is relatively weak. Could the authors address the strength of this interaction in the context of other export complexes?

*Indeed, the interaction between Imp13-RanGTP and eIF1A is rather weak. However, it falls in a similar range as the interactions of Crm1-RanGTP with most NESs (Askjaer et al., 1999; Paraskeva et al., 1999; Güttler et al., 2010). Other export factors such as Xpot and Exp4 display a higher affinity for their cargoes (of about one order of magnitude higher) (Lipowsky et al., 2000; Güttler et al., 2010). Crm1 also has a higher affinity for Snurportin, recognized through additional interactions (Paraskeva et al., 1999).*
*We added the information at page 13, were we now discuss the Kd measurements for Imp13 in the context of the other export complexes.*

2. Imp13 appears to be essential in vivo, but it is unclear if the viability defect can be attributed to any of the structurally characterized interactions. Do any of the mutations disrupting cargo binding affect viability?

*In order to address this question, we performed Imp13 knockdown in HeLa cell lines stably expressing either GFP, GFP-tagged Imp13 wild type (wt) or the described cargo-binding mutants (see attached figure to document the experiments performed, panels A-C). All cell lines expressed GFP-Imp13 at comparable levels and were growing at similar rates in the presence of the control siRNA (in panel A, C). However, every siRNA directed against Imp13 open reading frame impaired cell growth significantly (B). The same effect was obtained with three different siRNAs targeting different regions of Imp13 open reading frame (Figure 5D in the main text), used either singularly or in different combinations. Unfortunately, the expression of GFP-tagged Imp13 wt did not rescue the growth phenotype (in B, orange trace). One possible explanation for this could be the presence of the GFP tag: while we ensured that GFP-tagged Imp13 was still able to bind and transport the cargoes considered in this study, we can not exclude that the tag could impair association of other*

*cargoes, affecting other Imp13-mediated transport pathways. Another possibility could be that sequences other than Imp13 open reading frame are required. Indeed, in Drosophila larvae, lethality in Imp13 mutants could not be rescued by overexpressing Imp13 cDNA sequence alone (Giagtzoglou et al., 2009). In any case, this result makes it impossible to draw conclusions on the requirement of specific Imp13 transport pathways for cell viability.*

3. Overall, the manuscript contains a lot of redundant information and it should be considered to shorten the manuscript and focus on the new information.

*We carefully went through the manuscript and deleted redundant information we found.*

Minor comments

1. Only one methionine sidechain is visible in Figure S2A. Were the other two sidechains modeled? If so, they should be included in the figure. There is also a misspelling of methionine in the figure legend.

*The two methionine sidechains were modeled. We included the sidechains in Figure S2A and colored them to distinguish them from the only methionine sidechain visible in the electron density. We also corrected the typo, thank you for pointing this out.*

2. It would be nice to have an additional supplemental figure to complement the discussion comparing the Imp13 export complex to Crm1.

*Thank you for the suggestion. We add this figure as Fig. S7A-B and we refer to it in the text.*

3. The color/pattern choice for Figure 2D is not very easy to figure out. Could one of the green curves be given a pattern or could a different color scheme be used?

*As mentioned above, we now changed both the color and the pattern of the traces for better visibility.*

4. I am curious how the third copy of Imp13 bound only to RanGTP compares to the previously solved structure of Imp13-RanGTP. Does the dimer exhibit any conformational flexibility?

*Imp13 in the two structures displays a very similar conformation, suggesting that it is quite rigid in this state. We included a superposition of Imp13 in complex 3 and of Imp13 in the structure of the binary complex (pdb id.: 2x19) in figure S7B-C and we refer to it in the text.*

5. The color choices in the structure figures are not always ideal. In particular, the red and green shades which were used make it difficult for color blind readers and another color scheme should be considered.

*We changed the red/green into black or white/green in Fig. 4, 5D, 6B and S6D for better visibility.*

6. Please add the following information to Table 1: Total number of observations, number of unique observations in the last shell, redundancy in the last shell, total number of reflections used in refinement, number of reflections in the test set, number of refined atoms, number of solvent or ligand atoms, average B factors of protein and solvent atoms, and MolProbity score. Please include an appropriate reference for MolProbity.

*We completed the table with the information requested.*

7. Figure 7: please include the NPC in the figure and label the cytoplasm and nucleus.

*We included schematized NPCs and darkened the nucleus/cytoplasm labels for better visibility.*

Referee #3

In this study, the authors focused on Importin 13 (Imp 13) to know how Imp 13 can mediate both import and export of cargoes as a dual functional protein. They solved the crystal structures of Imp 13 in complex with RanGTP and one known export cargo eIF1A and the unbound state. They found that Imp 13 adopts a compact conformation with RanGTP where eIF1A recognizes the inner surface of Imp 13. Furthermore, it was shown that contacts on Imp 13 and on RanGTP stabilize export cargo binding. On the other hand, it has been shown that the association of Imp 13 with RanGTP suppresses that with the import cargo, indicating that the import cargo is released in the nucleus. The unbound form of Imp 13 revealed an open conformation, explaining export cargo release in the cytoplasm. In addition, they demonstrated that overexpression of Imp 13 induced cytoplasmic accumulation of eIF1A, meaning that Imp 13 exports eIF1A in vivo. They also observed that siRNA specific for Imp 13 showed a
significant decrease in cell viability, indicating that the function of Imp 13 is essential. Taken together, the authors propose that the combination of shape, charge and size of the bound cargo drives the concomitant or exclusive interactions with RanGTP and determines the directionality of the transport and the delivery of the cargoes in the appropriate compartment. Thus, the data in this study are convincing and interesting, and this study provides the structural basis to understand the dual functions of Imp 13. The following point should be addressed before publication.

In order to confirm the in vivo roles of Imp 13 on the import and export of proteins, the authors should examine the subcellular localization of endogenous Mago-Y14 and eIF1A proteins in the Imp 13-knockdown cells.

*Imp13-knockdown cells do not show a significant difference in the localization of endogenous Mago-Y14 and eIF1A when compared to control cells (See attached figure, panels E-H). However, endogenous Imp13 is still detectable in western blot, meaning that even in knockdown cells there is a remaining pool of Imp13 (I), which could be sufficient to transport the analyzed cargoes. Given the strong decrease in cell viability observed in Imp13-knockdown conditions, it is possible that the cells surviving the depletion procedure would be the ones which express sufficient Imp13 levels to perform the essential transport tasks. We also performed an alternative knockdown strategy, designed to obtain a more efficient Imp13-knockdown by transfecting HeLa cells with a plasmid encoding shRNAs targeting Imp13 open reading frame, and selecting for the shRNA-expressing cells with the appropriate antibiotic; however, no cell survived the selection process, further supporting the requirement of Imp13 for cell viability.*

(Please see figure at the end of this file.)

2nd Editorial Decision 16 January 2013

Thank you for submitting your revised manuscript for our consideration. I have now had a chance to look through it and to assess your responses to the comments raised by the original reviewers. I am very happy to inform you that we are ready to proceed with acceptance of the paper, pending modification of a few minor points.

- Please add the information about the statistical test used to create the error bars in Figure 4 to the Figure legend. In addition, please indicate the number of biological replicates performed to generate the data in Figure 5B as well as an analysis of the statistical significance.

- Please remember to add the accession numbers for the deposited structural data as soon as they are available.

- I would like to suggest changing the current title to "Structural basis for the nuclear export activity of Importin 13" as well as some minor alterations in the Abstract.

Importin13 (Imp13) is a bidirectional karyopherin that can mediate both import and export of cargoes. Imp13 recognizes several import cargoes, which include the exon junction complex components Mago-Y14 and the E2 SUMO-conjugating enzyme Ubc9, and one known export cargo, the translation initiation factor 1A (eIF1A). To understand how Imp13 can perform double duty, we determined the 3.6 Å crystal structure of Imp13 in complex with RanGTP and with eIF1A. eIF1A binds at the inner surface of the Imp13 C-terminal arch adjacent and concomitantly to RanGTP

illustrating how eIF1A can be exported by Imp13. Moreover, the 3.0 Å structure of Imp13 in its unbound state reveals the existence of an open conformation in the cytoplasm that explains export cargo release and completes the export branch of the Imp13 pathway. Finally, we demonstrate that Imp13 is able to bind and export eIF1A in vivo and that its function is essential.

- Please let me know if you would prefer that the data provided for reviewers should be omitted from the Review Process File that we publish with each article.

I will now return your manuscript to you for one additional round of minor revision. After that we should be able to swiftly proceed with formal acceptance and production of the manuscript!

If you have any questions, please do not hesitate to contact me directly.

2nd Revision - authors' response 28 January 2013

- Please add the information about the statistical test used to create the error bars in Figure 4 to the Figure legend. In addition, please indicate the number of biological replicates performed to generate the data in Figure 5B as well as an analysis of the statistical significance.

*Figure 4: we included information about statistical analysis in the figure legend. We also removed outliers in the figure to simplify data representation.*
*Figure 5: we included information on experimental replicates and statistical significance both in the figure and in the figure legend.*
*Supplementary Fig. 4 and 5: we included statistical analysis and removed outliers.*

- Please remember to add the accession numbers for the deposited structural data as soon as they are available.

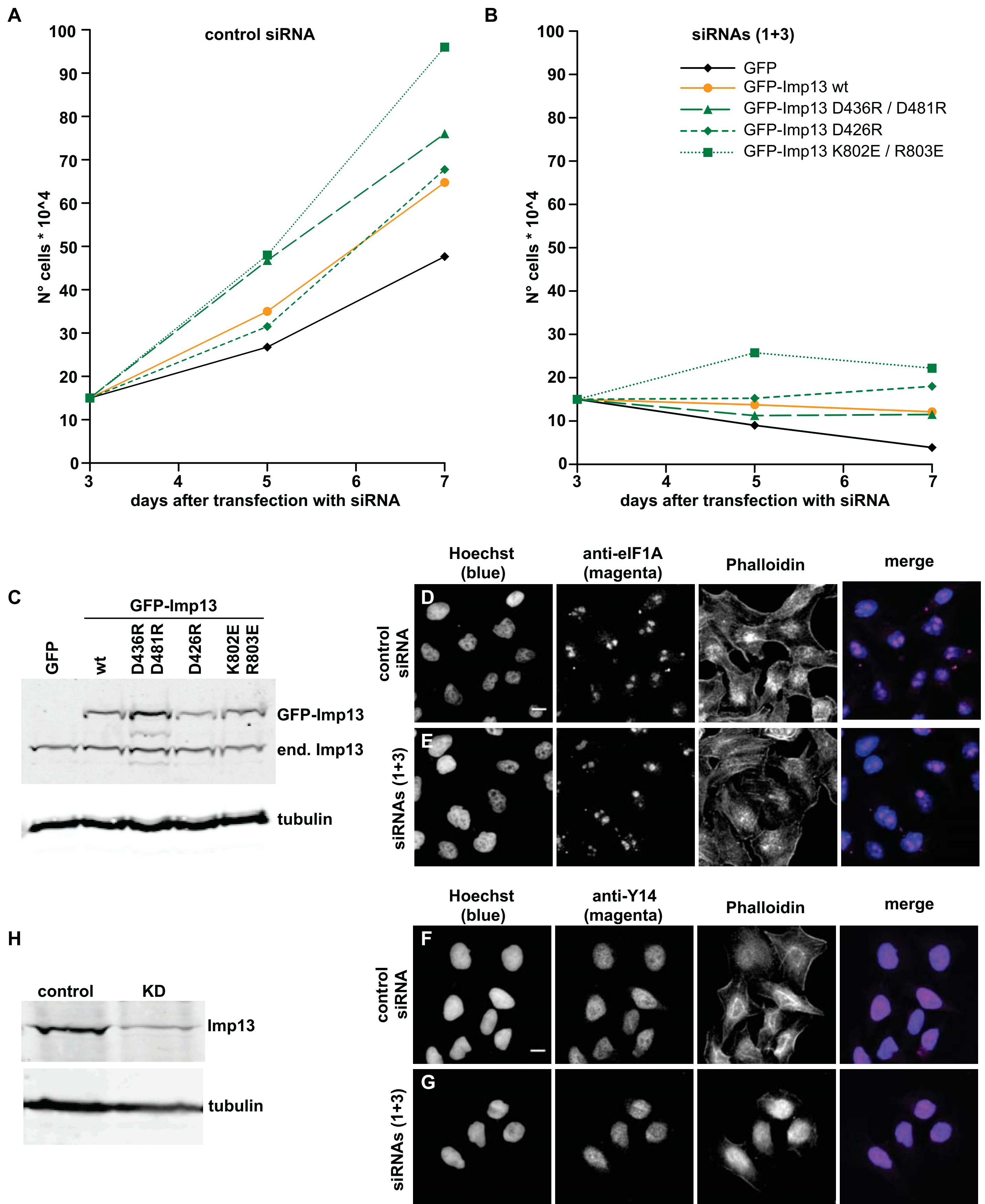
*Done.*

- I would like to suggest changing the current title to "Structural basis for the nuclear export activity of Importin 13" as well as some minor alterations in the Abstract.

*The changes you suggest are fine with us.*

- Please let me know if you would prefer that the data provided for reviewers should be omitted from the Review Process File that we publish with each article.

*I think the data can be kept together with the review process file.*




**A**-**B**) HeLa cells stably expressing GFP, GFP-tagged Imp13 wt or the indicated mutants were transfected with a control siRNA (**A**) or siRNAs targeting Imp13 (**B**). Cell numbers were determined up to 7 days after the first transfection; at day 3 the cells were re-seeded for a second transfection with the same siRNAs (see also matherials and methods). (**C**) Western blot showing the expression levels of endogenous and GFP-tagged Imp13 wt or the indicated mutants in the stable cell lines used for the knockdown experiments; the GFP-tagged constructs are expressed at levels comparable to that of endogenous Imp13. The efficiency of endogenous Imp13 knockdown for the indicated siRNAs combinations is shown in Fig. 5C. (**D**-**E**) Subcellular localization of endogenous eIF1A in HeLa cells transfected either with a control siRNA (**D**) or with siRNAs targeting Imp13 (**E**). (**F**-**G**) Subcellular localization of endogenous Y14 in HeLa cells transfected either with a control siRNA (**F**) or with siRNAs targeting Imp13 (**G**); rabbit anti-Y14 serum was kindly provided by Elisa Izaurralde. (**H**) Western blot showing the efficiency of endogenous Imp13 knockdown in the cells used for the immunostainings (**D**-**G**).