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Human SFI1 and centrin form a complex critical for centriole architecture and ciliogenesis

Marine Laporte, Imene Bouhlel, Eloise Bertiaux, Ciaran Morrison, Alexia Giroud, Susanne Borgers, Juliette Azimzadeh, Michel Bornens, Paul Guichard, Anne Paoletti and Virginie Hamel
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Review #1

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

It is shown that SFI1 in humans is a bona fide centrosome protein that localizes to the distal end of the centriolar lumen, where it co-localizes with a distal pool of centrins 2 + 3. A second pool of these centrins is found at the inner centriolar core. Depletion of SFI1 by siRNA leads to loss of the distal centrin pool, whereas depletion of POC5 specifically removes centrins from the core. Moreover, depletion of SFI1 affects the integrity of the centriolar wall, provokes the loss of radial centriolar symmetry, and inhibits ciliogenesis, but does not prevent centriolar duplication.

This is a solid study that characterizes the localization of SFI1 and its interaction with centrins 2 + 3. The microscopy images are of high quality (ultrastructure expansion microscopy). The manuscript has a descriptive character; the information on SFI1-dependent cellular mechanisms is limited, but nevertheless of high potential interest to a readership working on the assembly of centrosomes and cilia.

Suggestions for additional improvements:

- Additional information on the efficiency of SFI1 depletion should be given: SFI1-positive/negative centrioles are scored in Figure 3I, but was there a threshold for the detection of SFI1-immunofluorescence signal, or was there a range of measured SFI1 signal? What was the transfection efficiency of Sfi1-siRNA?
- In Figure 1M, the relative position of SFI1 versus centrins 2 + 3 is determined indirectly, by measuring the position relative to the alpha/beta-tubulin signal of the centriolar cylinders. Would it be possible to perform U-ExM on centrioles stained for SFI1 in combination with centrins 2 + 3 (such as in Figure 1B), to verify direct co-localization?
- The proposed role of SFI1 in centriole duplication by Balestra et al (2013) and Kodani et al (2019) conflicts with the findings in this manuscript, where no effect on duplication is seen after depletion. Unfortunately, the authors make little effort to reconcile the different findings in their discussion, even though the explanation may be trivial: the previous studies used centrin immunofluorescence to monitor duplication of centrioles, and since centrin signal is reduced after SFI1-depletion (loss of distal centrin), previous studies might have erroneously interpreted this as 'lack of centriole duplication'. I suggest that this should be better explained in the discussion, to resolve the potential conflict.

2. Significance:

Significance (Required)

This is a solid study that characterizes the localization of SFI1 and its interaction with centrins 2 + 3. The microscopy images are of high quality (ultrastructure expansion microscopy). The manuscript has a descriptive character; the information on SFI1-dependent cellular mechanisms is limited, but nevertheless of high potential interest to a readership working on the assembly of centrosomes and cilia.

Reviewer's expertise:

centrosomes, microtubules, microscopy, cell biology)

Referee Cross-commenting

I think that all reviewers have some valid points. If the authors aimed for impact, they would be well advised to revise their manuscript to the best of their abilities, following the reviewers' suggestions as a guide. Otherwise, the manuscript may be published as a descriptive study, but remaining less attractive to non-specialists.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

Review #2

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

In this manuscript, Bouhlel et al. analyzed the molecular function of SFI1, a conserved centriole component from yeast, in centriole formation. Using super resolution microscopy, the authors demonstrated that SFI1 localizes to the inner lumen of the very distal end of centrioles. SFI1 colocalized with a pool of centrin proteins there, and the complex was detectable at the distal end from the early stage of centriole formation. Depletion of SFI1 leads to removal of this pool of centrin proteins from the distal end. This regulation seems to be different from that by POC5. Although depletion of SFI1 did not affect centriole duplication by monitoring the localization of procentriole proteins, it perturbed the formation of distal tip of daughter centrioles. Maybe reflecting this phenotype, ciliogenesis was significantly suppressed in the absence of SFI1.

Overall, in this manuscript, the quality of data is convincing, and the manuscript is well written. This study can be of great interest to the centrosome field and also to cell biologists. Nevertheless, I would raise some points that the authors could experimentally address.

Major points:

1. To clarify the cause of ciliogenesis defects in SFI1 knock-down cells, the integrity of distal appendages should be examined. It is possible that the lack of distal appendages is a cause of the phenotype in ciliogenesis.
2. The structural defects of centrioles by SFI1 depletion is very interesting. It would be informative if the authors could further analyze which proteins are reduced at the distal end of centrioles by SFI1 depletion. For example, did the authors check WDR90 and related distal end proteins?
3. I am wondering whether the defects in the centriole integrity by SFI1 depletion are due to lack of stability or compromised formation. This can be addressed by gathering various stages of elongating centrioles in SFI1 knock-down cells.

Minor points:

4. How would SFI1 proteins, in the center of distal ends, regulate the formation/stability of the centriole wall? There should be a substantial distance, so other proteins may bridge in between. This point should be more thoroughly discussed in the text.
5. The observation that SFI1 is loaded onto the distal end of elongating centrioles in the very early stage is interesting. What would be the possible recruiters/regulators? This can be discussed in the text.

2. Significance:

Significance (Required)

This study provides evidence that a conserved factor SFI1 also functions in centriole formation in human. This protein ensures proper centriole growth and its structural integrity, which is also critical for ciliogenesis. Thus, this study helps our understanding of centriole biogenesis across evolution.

This kind of conserved module functioning in both centrioles and spindle pole bodies is rare. So, this study will be of great interest to researchers in centrosome research like this reviewer.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

Review #3

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The interacting centrin (Cdc31 in yeast) and Sfi1 are conserved proteins of microtubule organizing centres. Best know is the yeast Sfi1/Cdc31 system. Sfi1 and Cdc31 are essential for spindle pole body duplication as components of the half bridge and bridge. Binding of Cdc31 to Sfi1 has at least three functional roles: 1) Cdc31 stabilizes the Sfi1 structure; 2) Cdc31 mediates lateral interactions between Sfi1-Cdc31 filaments; 3) N-terminal Cdc31 binding sites in Sfi1 are important for satellite formation. In contrast, the role of the SFI1/Centrin complex at human centrosomes is much less understood although Kilmartin (J Cell Biol) already reported in 2003 that human centrosomes contain Sfi1. Since then Kodani et al reported (J Cell Biol) that human SFI1 interacts with deubiquitinase USP9X to protect STIL from degradation and to promote centriole duplication. In addition, these authors report a rather undefined centriolar localisation of human SFI1.

In this newly submitted publication, Bouhtel et al perform expansion microscopy and define the localisation of

SFI1 and centrins in human RPE1 and U2OS cells. This resulted in beautiful images describing that SFI1 localises dot like at the distal end of centrioles where it forms a complex with centrin 2 and 3. Centrin 2 and 3 have an additional, more central localisation inside centrioles where they interact with POC5 as previously reported. siRNA depletion of SFI1 or POC5 affect the respective centrin pools. In addition, the authors show that SFI1 is not involved in centriole duplication as published by Kodani et al but instead has a role in cilia formation. However, this function of SFI1 was not thoroughly analysed - it remains unclear whether defects in centriole structure probably indirectly affect cilia formation or whether SFI1 has a more direct role in for example recruitment of ciliary vesicles (the authors favour the first explanation - however the cilia defect (>50% reduced compared to WT) is more pronounced than the centriole defect (35%)). Finally, the authors describe based on expansion microscopy defects in centriole morphology. Below I have listed specific points that should be addressed by the authors before publication of this paper.

Specific comments

1. New literature on yeast Sfi1 and satellite formation is not referenced.
2. Kodani and Bouhtel describe different localisations and functions of SFI1. It is like that they studied different proteins! I find it important for the community to perform addition experiments and rescue controls in order to get to the bottom of these discrepancies. Kodani et al mainly used HeLa cells. Bouhtel et al used U2OS cells. Bouhtel et al use siRNA from Eurogentec; Kodani et al from Life Technologies. Bouhtel et al should compare the outcome of siRNA depletion of SFI1 in HeLa and U2OS cells using both siRNAs (Fig. 4I and J). Rescue controls for some phenotypes are also needed. This comparison will give the authors the chance to discuss the differences between both publications more thoroughly (cell lines, depletion efficiencies, siRNA, technical differences).
3. Fig. 5H shows POC5-Centrin as spiral. Is this a model or do the authors have data that support such localisation? I would modify Fig. 5H to what the expansion microscopy shows.
4. The SFI1 depletion phenotype on centrioles is quite variable (Fig. 5B). About half of the centrioles are of normal roundness, others are less round. How do the authors explain this variation? How do the authors explain the only partial centriole MT defect (35%) in Fig. 5F? Is there a correlation between SFI1 depletion efficiency and the strength of the phenotype.
5. The authors state in Fig. S2B, C, F and H that a Sfi1 signal appeared in a more proximal region of the centriole and that this signal is decreasing after Sfi1 depletion. Why did the authors not investigate this second SFI1 pool?

2. Significance:

Significance (Required)

The manuscript determines convincingly the localisation of SFI1, centrin 2 and 3 and clearly shows how centrin is recruited to distinct sites within centrioles by POC5 and SFI1. However, because of the lack of functional data, the overall impact of the manuscript is limited.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 3 and 6 months

Full Revision

Manuscript number: RC- 2021-01088

Corresponding author(s): Paul Guichard, Anne Paoletti and Virginie Hamel.

1. General Statements [optional]

This section is optional. Insert here any general statements you wish to make about the goal of the study or about the reviews.

2. Point-by-point description of the revisions

We would like to thank Review Commons for efficient editorial processing and appreciate the Reviewers' comments and suggestions. We have made all efforts to address the comments and suggestions and detail our replies in the point-by-point response below. Note that for the sake of clarity and to facilitate the evaluations, we have highlighted changes in the text in blue in the manuscript.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

It is shown that SFI1 in humans is a bona fide centrosome protein that localizes to the distal end of the centriolar lumen, where it co-localizes with a distal pool of centrins 2 + 3. A second pool of these centrins is found at the inner centriolar core. Depletion of SFI1 by siRNA leads to loss of the distal centrin pool, whereas depletion of POC5 specifically removes centrins from the core. Moreover, depletion of SFI1 affects the integrity of the centriolar wall, provokes the loss of radial centriolar symmetry, and inhibits ciliogenesis, but does not prevent centriolar duplication.

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We thank the reviewer for careful reading of the manuscript and his/her supporting comments and suggestions to further improve the quality of our manuscript. With our new results, we hope that the reviewer will find that our manuscript now clearly identifies SFI1-dependent cellular mechanisms.
Suggestions for additional improvements:
- Additional information on the efficiency of SFI1 depletion should be given: SFI1-positive/negative centrioles are scored in Figure 3I, but was there a threshold for the detection of SFI1-immunofluorescence signal, or was there a range of measured SFI1 signal? What was the transfection efficiency of Sfi1-siRNA?
We apologize for not being sufficiently clear in the first instance. We now provide additional detail on the efficiency of SFI1 depletion in the Materials and Methods section (p.24 of the revised manuscript). Only SFI1-negative centrioles were counted as depleted. All the quantifications were performed on pictures where we set brightness to its maximum to ensure that centrioles counted as depleted were indeed totally devoid of SFI1 signal. Maximum intensity images are now presented in Figure S2L, M to illustrate and clarify this

important point. We did not count centrioles in which the SFI1 signal was still visible, even if reduced in intensity, as SFI1 negative. The estimated transfection efficiency of SFI1 siRNA was high, as witnessed by an 87% depletion of centriolar SFI1 in U2OS, 89 % in HeLa and 91% in RPE-1 cells. These numbers reflect the percentage of cells where the SFI1 signal was missing on at least one of the centrioles from a centrosome pair (partial SFI1 depletion: 35%, 33% and 40% for U2OS, HeLa and RPE-1 cells, respectively) and where SFI1 was entirely depleted from the centrosome pair (total SFI1 depletion: 52%, 56%, 51% for U2OS, HeLa and RPE-1 cells, respectively). Please note that the graphs of Figure 3 have been updated and now represent the proportion of cells presenting intact SFI1, partial SFI1 and no SFI1 signals.

- In Figure 1M, the relative position of SFI1 versus centrins 2 + 3 is determined indirectly, by measuring the position relative to the alpha/beta-tubulin signal of the centriolar cylinders. Would it be possible to perform U-ExM on centrioles stained for SFI1 in combination with centrins 2 + 3 (such as in Figure 1B), to verify direct co-localization?
This is a great suggestion to directly visualize the co-localization of the SFI1/Centrin2/3 complex. Following the reviewer's suggestion, we performed a triple staining for tubulin, SFI1 and Centrin2/3. Consistent with our previous data, we found that SFI1 and Centrin2/3 co-localize, with a measured SFI1-Centrin2/3 average distance of 35 nm. These new data are now presented in Figure 1I, L and described in p. 7 of the revised manuscript.

- The proposed role of SFI1 in centriole duplication by Balestra et al (2013) and Kodani et al (2019) conflicts with the findings in this manuscript, where no effect on duplication is seen after depletion. Unfortunately, the authors make little effort to reconcile the different findings in their discussion, even though the explanation may be trivial: the previous studies used centrin immunofluorescence to monitor duplication of centrioles, and since centrin signal is reduced after SFI1-depletion (loss of distal centrin), previous studies might have erroneously interpreted this as 'lack of centriole duplication'. I suggest that this should be better explained in the discussion, to resolve the potential conflict.
We apologize here for our lack of clarity regarding the conflicting findings between our study and that of Kodani et al. (2019). Indeed, as proposed by the reviewer, one of the explanations for the misinterpretation of the results by Kodani et al. on the duplication phenotype might have been based on the loss of Centrin. To clarify this, we now refer to it in the revised manuscript p. 10, 11 and 16.
In addition, and as requested by Reviewer 3, we experimentally tested the apparent discrepancy regarding the reported SFI1 phenotypes. Since the two studies used different siRNAs and different cell lines, we monitored SFI1 depletion and its associated phenotypes using our siRNA (siRNA#A; also used in Balestra et al.) and the Kodani et al. siRNA (siRNA#B) in both HeLa and U2OS cell lines. Importantly, we demonstrate that our siRNA (siRNA#A) displays similar SFI1-depletion efficiency in both cell lines, recapitulating the same phenotypes: loss of distal centrin (86%), abnormal centrioles (21%) and no duplication defects. In contrast, we found that the siRNA#B (Kodani et al, 2019) used following the authors' published experimental conditions led to a weaker SFI1 depletion both in U2OS and HeLa cells. Despite the lack of full SFI1 depletion at centrioles, we recapitulated the reduced SFI1 signal intensity at centrioles (30-40%). Consistent with our data, the reduced SFI1 levels mirrors reduced Centrin levels at centrioles. However, we did not observe duplication defects or abnormal centrioles. We hypothesize that the lack of abnormal centrioles is inherent to the remaining Centrin signals at centrioles, notably at the level of the inner scaffold that would protect it from structural defects. In conclusion, we now clearly specify that the putative duplication defects upon SFI1 depletion most likely result from a misinterpretation of the loss or reduced Centrin signal

originally reported in the Kodani et al paper (see discussion p.16). These new data are now reported in Figure S5 of the revised manuscript.
Finally, to fully ascertain the specificity of the SFI1 depletion phenotypes that we report in this manuscript, we performed rescue experiments, as requested by Reviewer 3. We initially intended to rescue the phenotype of SFI1-depletion by exogenous expression of different tagged version of SFI1 already available in the community (GFP-SFI1, mcherry-SFI1, SNAP-SFI1). Unfortunately, we found that none of these SFI1-tagged proteins localized properly at centrioles (Figure S4) and therefore were not expected to rescue SFI1 depletion. Based on these results, we hypothesized that tagging SFI1 might be deleterious for its proper localization and function and, instead, cloned an untagged SFI1 RNAi-resistant version (SFI1-RR) in a pIRES-GFP plasmid allowing for the expression of SFI1-RR and GFP as separated proteins. Importantly, we demonstrate that expression of SFI1-RR in SFI1-depleted U2OS cells rescues SFI1 and Centrin localization at the distal end of the centriole (Figure 3K-N) as well as the structural defects at the levels of centrioles (Figure 4O-P). Moreover, we also performed a rescue experiment in ciliated RPE1 cells and demonstrated that SFI1-RR expression rescued ciliogenesis and CP110 uncapping to the level of control cells (Figure 5L-N). Finally, to complete further the study of SFI1 function, we analyzed knockout cells of its complex partner Centrin 2, generated by the Morrison lab (Prosser and Morrison, JCB, 2015). Importantly, we found that in Centrin2 KO RPE-1 cells, which have ciliogenesis defects similar to SFI1-depleted cells, SFI1 is no longer localized as a distal dot at centrioles, demonstrating that both proteins are interdependent for their localization at the distal end of centrioles. Moreover, we show that upon Centrin2 rescue in the Centrin2 KO cells, SFI1 localization as a distal dot is restored, demonstrating the specificity of the complex at that location. Taken together, we believe that our study now unambiguously defines the role of SFI1 at centrioles and addresses the technical discrepancies that concerned the referees.

Reviewer #1 (Significance (Required)):

This is a solid study that characterizes the localization of SFI1 and its interaction with centrins 2 + 3. The microscopy images are of high quality (ultrastructure expansion microscopy). The manuscript has a descriptive character; the information on SFI1-dependent cellular mechanisms is limited, but nevertheless of high potential interest to a readership working on the assembly of centrosomes and cilia.

(Reviewer's expertise:
centrosomes, microtubules, microscopy, cell biology)

****Referee Cross-commenting****

I think that all reviewers have some valid points. If the authors aimed for impact, they would be well advised to revise their manuscript to the best of their abilities, following the reviewers' suggestions as a guide. Otherwise, the manuscript may be published as a descriptive study, but remaining less attractive to non-specialists.
We thank the reviewer for his opinion and decided to revise our manuscript following the comments of all reviewers. We believe that the revised manuscript is greatly improved by the revisions, and we now provide a more mechanistic study of the role of SFI1 at centrioles and notably during ciliogenesis. We hope that the reviewer will appreciate the additional experiments.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

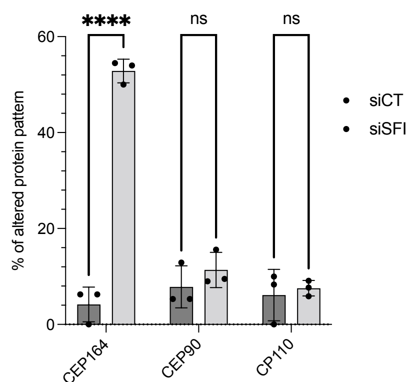
In this manuscript, Bouhlel et al. analyzed the molecular function of SFI1, a conserved centriole component from yeast, in centriole formation. Using super resolution microscopy, the authors demonstrated that SFI1 localizes to the inner lumen of the very distal end of centrioles. SFI1 colocalized with a pool of centrin proteins there, and the complex was detectable at the distal end from the early stage of centriole formation. Depletion of SFI1 leads to removal of this pool of centrin proteins from the distal end. This regulation seems to be different from that by POC5. Although depletion of SFI1 did not affect centriole duplication by monitoring the localization of procentriole proteins, it perturbed the formation of distal tip of daughter centrioles. Maybe reflecting this phenotype, ciliogenesis was significantly suppressed in the absence of SFI1. Overall, in this manuscript, the quality of data is convincing, and the manuscript is well written. This study can be of great interest to the centrosome field and also to cell biologists.
We thank the reviewer for careful reading of the manuscript and his/her supporting comments and suggestions to further improve the quality of our manuscript.

Nevertheless, I would raise some points that the authors could experimentally address.
Major points:
1. To clarify the cause of ciliogenesis defects in SFI1 knock-down cells, the integrity of distal appendages should be examined. It is possible that the lack of distal appendages is a cause of the phenotype in ciliogenesis.
We thank the reviewer for this good suggestion, which we address in the revised version of the manuscript. To investigate whether distal appendages proteins were affected upon SFI1 depletion, we analyzed their distribution by expansion microscopy (U-ExM) in RPE-1 cells undergoing ciliogenesis. Importantly, we found that the localization of the distal centriolar protein CP110, a negative regulator of ciliogenesis, was affected upon SFI1 depletion. CP110 normally caps the distal ends of centrioles in cycling cells. At the onset of ciliogenesis, CP110 is removed from the mother centriole distal end to allow cilium formation, while remaining on the daughter centriole (see Figure 5I as an illustration). Upon ciliogenesis, 47% of SFI1 siRNA-treated RPE-1 cells retained CP110 at the distal ends of mother centrioles, while 90% of control cells had correctly removed CP110 from the mother centrioles. Importantly, we could rescue both ciliogenesis defects and CP110 uncapping by adding back untagged RNAi-resistant SFI1, indicating that the SFI1/Centrin complex at centrioles regulates ciliogenesis partly by controlling CP110 at centrioles distal ends. This data is consistent with the reported function of Centrin2 in ciliogenesis (Prosser and Morrison, JCB, 2015). Interestingly, we could also recapitulate the phenotype of structurally abnormal centrioles upon SFI1 depletion in RPE-1 cells, similarly to what we found in U2OS and HeLa cells. These new data are now reported in Figure 5.
To further dissect the function of SFI1 in ciliogenesis, we analyzed the distribution of the distal appendage marker, Cep164, as it has been shown to be partly affected in Centrin2 knock-out cells (Prosser and Morrison, JCB, 2015). Consistent with the published phenotype, we found that the canonical 9-fold symmetrical pattern of Cep164 was disturbed in the absence of SFI1. Intriguingly, the CEP164 defects were not correlated to the structurally abnormal centrioles, indicating that this defect is not due to the loss of centriolar integrity. This is now reported in Figure 6 and Figure S7. In addition, we monitored the distribution of the distal centriolar protein CEP90, which has been recently proposed to specify the location and assembly of distal appendages (Kumar et al, JCB, 2021 and Le Borgne, BioRxiv, 2021). We found that CEP90

distribution was not affected upon SFI1 depletion, indicating that the CEP164 defects are probably not due to a problem in CEP90-dependent recruitment (Figure 6).

2. The structural defects of centrioles by SFI1 depletion is very interesting. It would be informative if the authors could further analyze which proteins are reduced at the distal end of centrioles by SFI1 depletion. For example, did the authors check WDR90 and related distal end proteins?

This interesting point is somewhat related to the previous comment on the ciliogenesis defects and the impact on distal proteins. As stated above, we monitored the distribution of distal and distal appendage proteins: CEP90, CP110 and CEP164 upon SFI1 depletion. While we observed CP110 decapping defects during ciliogenesis, we found that CP110 distribution was not altered in cycling cells (see graph below and Figure S6). Similarly, we did not observe any localization defects for CEP90.



As reported above, the observed CEP164 distribution defects were not correlated to the structurally abnormal centrioles, indicating that this defect is not due to the loss of centriolar integrity (Figure S7). We did not analyze WDR90 distribution since it has been reported as a central core protein, similar to POC5 and Centrin that we analyzed already in the initial manuscript.

3. I am wondering whether the defects in the centriole integrity by SFI1 depletion are due to lack of stability or compromised formation. This can be addressed by gathering various stages of elongating centrioles in SFI1 knock-down cells.

This is a very interesting point that is challenging to address. To answer this point, we captured top-viewed growing procentrioles and quantified the roundness index and general aspect (open / broken procentrioles). We found no difference between control and SFI1-depleted procentrioles, suggesting that the structural defects that we observed are not occurring during centriole assembly. This new result indicates that it is rather the overall stability of mature centrioles that is altered upon SFI1 depletion. These new data are now reported in Figure 4 K, L and described in p. 12 of the revised manuscript.

Minor points:

4. How would SFI1 proteins, in the center of distal ends, regulate the formation/stability of the centriole wall? There should be a substantial distance, so other proteins may bridge in between. This point should be more thoroughly discussed in the text.

One possibility would be that we are looking at the C-terminal portion of SFI1 and that the N-terminal part of the protein could be located closer to the microtubule wall. However, this hypothesis cannot be tested

owing to the lack of appropriate antibodies. We stated this hypothesis in the Discussion (p. 17 of the revised manuscript).
A second hypothesis would be that one or several other proteins bridge SFI1 to the microtubule wall. Centrin could be a good candidate as it is not only as a distal dot but it is also closer to the microtubule wall in the central core region. It is important to note that another, unknown protein(s) could also be making this link. These hypotheses are now clearly stated in the discussion (p. 17 of the revised manuscript).

5. The observation that SFI1 is loaded onto the distal end of elongating centrioles in the very early stage is interesting. What would be the possible recruiters/regulators? This can be discussed in the text.
We think that Centrin might be the possible recruiter as it is also loaded in the very early stages of centriole assembly. However, it is difficult to know which one of Centrin or SFI1 is the first as we now demonstrate that both proteins are interdependent for their localizations at the centriolar distal end. Indeed, we have included an analysis of SFI1 distribution in Centrin-2 KO RPE-1 cells generated by the Morrison lab (Prosser and Morrison, JCB, 2015), by U-ExM. We found that SFI1 localization at the distal region is dependent on Centrin-2. Moreover, we demonstrate that addition of Centrin-2 can restore SFI1 distal localization. This new set of data is now provided in Figure 3O, P and referred to in the revised manuscript p.9, 10.

Reviewer #2 (Significance (Required)):

This study provides evidence that a conserved factor SFI1 also functions in centriole formation in human. This protein ensures proper centriole growth and its structural integrity, which is also critical for ciliogenesis. Thus, this study helps our understanding of centriole biogenesis across evolution.
This kind of conserved module functioning in both centrioles and spindle pole bodies is rare. So, this study will be of great interest to researchers in centrosome research like this reviewer.

We thank the reviewer for this supportive comment. We hope that the additional experiments now presented in the revised version of the manuscript will even more reinforce the impact of our study.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

The interacting centrin (Cdc31 in yeast) and Sfi1 are conserved proteins of microtubule organizing centres. Best know is the yeast Sfi1/Cdc31 system. Sfi1 and Cdc31 are essential for spindle pole body duplication as components of the half bridge and bridge. Binding of Cdc31 to Sfi1 has at least three functional roles: 1) Cdc31 stabilizes the Sfi1 structure; 2) Cdc31 mediates lateral interactions between Sfi1-Cdc31 filaments; 3) N-terminal Cdc31 binding sites in Sfi1 are important for satellite formation. In contrast, the role of the SFI1/Centrin complex at human centrosomes is much less understood although Kilmartin (J Cell Biol) already reported in 2003 that human centrosomes contain Sfi1. Since then Kodani et al reported (J Cell Biol) that human SFI1 interacts with deubiquitinase USP9X to protect STIL from degradation and to promote centriole duplication. In addition, these authors report a rather undefined centriolar localisation of human SFI1.
In this newly submitted publication, Bouhtel et al perform expansion microscopy and define the localisation of SFI1 and centrins in human RPE1 and U2OS cells. This resulted in beautiful images describing that SFI1 localises dot like at the distal end of centrioles where it forms a complex with centrin 2 and 3. Centrin 2 and 3 have an additional, more central localisation inside centrioles where they interact with POC5 as previously

reported. siRNA depletion of SFI1 or POC5 affect the respective centrin pools. In addition, the authors show that SFI1 is not involved in centriole duplication as published by Kodani et al but instead has a role in cilia formation. However, this function of SFI1 was not thoroughly analysed - it remains unclear whether defects in centriole structure probably indirectly affect cilia formation or whether SFI1 has a more direct role in for example recruitment of ciliary vesicles (the authors favour the first explanation - however the cilia defect (>50% reduced compared to WT) is more pronounced than the centriole defect (35%)). Finally, the authors describe based on expansion microscopy defects in centriole morphology. Below I have listed specific points that should be addressed by the authors before publication of this paper.
We thank the reviewer for his/her careful reading of our manuscript and for his/her comments that we followed and think greatly improved our manuscript.

Specific comments

1. New literature on yeast Sfi1 and satellite formation is not referenced.
We apologize for this omission. We have now added the missing references (p. 4-5 of the revised manuscript).

2. Kodani and Bouhtel describe different localisations and functions of SFI1. It is like that they studied different proteins! I find it important for the community to perform addition experiments and rescue controls in order to get to the bottom of these discrepancies. Kodani et al mainly used HeLa cells. Bouhtel et al used U2OS cells. Bouhtel et al use siRNA from Eurogentec; Kodani et al from Life Technologies. Bouhtel et al should compare the outcome of siRNA depletion of SFI1 in HeLa and U2OS cells using both siRNAs (Fig. 4I and J). Rescue controls for some phenotypes are also needed. This comparison will give the authors the chance to discuss the differences between both publications more thoroughly (cell lines, depletion efficiencies, siRNA, technical differences).
We understand that explaining the discrepancies between our study and that of Kodani et al. (2019) will be very useful for the community, a point also raised by Reviewer 1.
Since the two studies used different siRNAs and different cell lines, we monitored SFI1 depletion and its associated phenotypes using our siRNA (siRNA#A; also used in Balestra et al.) and the Kodani et al. siRNA (siRNA#B) in both HeLa and U2OS cell lines. Importantly, we demonstrate that our siRNA (siRNA#A) displays similar SFI1-depletion efficiency in both cell lines, recapitulating the same phenotypes: loss of distal centrin (86%), abnormal centrioles (21%) and no duplication defects. In contrast, we found that the siRNA#B (Kodani et al, 2019) used following the authors' published experimental conditions led to a weaker SFI1 depletion both in U2OS and HeLa cells. Despite the lack of full SFI1 depletion at centrioles, we recapitulated the reduced SFI1 signal intensity at centrioles (30-40%). Consistent with our data, the reduced SFI1 levels mirrors reduced Centrin levels at centrioles. However, we did not observe duplication defects or abnormal centrioles. We hypothesize that the lack of abnormal centrioles is inherent to the remaining Centrin signals at centrioles, notably at the level of the inner scaffold that would protect it from structural defects. In conclusion, we now clearly specify that the putative duplication defects upon SFI1 depletion most likely result from a misinterpretation of the loss or reduced Centrin signal originally reported in the Kodani et al paper (see discussion p.16). These new data are now reported in Figure S5 of the revised manuscript. Finally, to fully ascertain the specificity of the SFI1 depletion phenotypes that we report in this manuscript, we performed rescue experiments, as requested by the reviewer.

We initially intended to rescue the phenotype of SFI1-depletion by exogenous expression of different tagged version of SFI1 already available in the community (GFP-SFI1, mcherry-SFI1, SNAP-SFI1). Unfortunately, we found that none of these SFI1-tagged proteins localized properly at centrioles (Figure S4) and therefore were not expected to rescue SFI1 depletion. Based on these results, we hypothesized that tagging SFI1 might be deleterious for its proper localization and function and, instead, cloned an untagged SFI1 RNAi-resistant version (SFI1-RR) in a pIRES-GFP plasmid allowing for the expression of SFI1-RR and GFP as separated proteins. Importantly, we demonstrate that expression of SFI1-RR in SFI1-depleted U2OS cells rescues SFI1 and Centrin localization at the distal end of the centriole (Figure 3K-N) as well as the structural defects at the levels of centrioles (Figure 4O-P). Moreover, we also performed a rescue experiment in ciliated RPE1 cells and demonstrated that SFI1-RR expression rescued ciliogenesis and CP110 uncapping to the level of control cells (Figure 5L-N). Finally, to complete further the study of SFI1 function, we analyzed knockout cells of its complex partner Centrin 2, generated by the Morrison lab (Prosser and Morrison, JCB, 2015). Importantly, we found that in Centrin2 KO RPE-1 cells, which have ciliogenesis defects similar to SFI1-depleted cells, SFI1 is no longer localized as a distal dot at centrioles, demonstrating that both proteins are interdependent for their localization at the distal end of centrioles. Moreover, we show that upon Centrin2 rescue in the Centrin2 KO cells, SFI1 localization as a distal dot is restored, demonstrating the specificity of the complex at that location.
Taken together, we believe that our study now unambiguously defines the role of SFI1 at centrioles and addresses the technical discrepancies that concerned the referees.

3. Fig. 5H shows POC5-Centrin as spiral. Is this a model or do the authors have data that support such localisation? I would modify Fig. 5H to what the expansion microscopy shows.
We understand the reviewer's comment. Figure 5H is a model based on what we know from cryo-EM data about the inner scaffold. However, it is true that by U-ExM we do not have a sufficient resolution to see a spiral signal. We therefore modified the model and simplified it to fit the U-ExM staining as suggested.

4. The SFI1 depletion phenotype on centrioles is quite variable (Fig. 5B). About half of the centrioles are of normal roundness, others are less round. How do the authors explain this variation? How do the authors explain the only partial centriole MT defect (35%) in Fig. 5F? Is there a correlation between SFI1 depletion efficiency and the strength of the phenotype.
SFI1 depletion by siRNA is very robust with about 90% of depleted centrioles in the three tested cell lines. As mentioned in the response to Reviewer 1, only SFI1-negative centrioles were counted as depleted. All the quantifications were performed on pictures where we set brightness to its maximum to ensure that centrioles counted as depleted were indeed totally devoid of SFI1 signal. Maximum intensity images are now presented in Figure S2L, M to illustrate and clarify this important point. We did not count centrioles in which the SFI1 signal was still visible, even if reduced in intensity, as SFI1 negative. The estimated transfection efficiency of SFI1 siRNA was high as witnessed by an 87% depletion of centriolar SFI1 in U2OS, 89 % in HeLa and 91% in RPE-1 cells. These numbers reflect the percentage of cells where the SFI1 signal was missing on at least one of the centrioles from a centrosome pair (partial SFI1 depletion: 35%, 33% and 40% for U2OS, HeLa and RPE-1 cells, respectively) and where SFI1 was entirely depleted from the centrosome pair (total SFI1 depletion: 52%, 56%, 51% for U2OS, HeLa and RPE-1 cells, respectively). Please note that the graphs of Figure 3 have been updated and now represent the proportion of cells presenting intact SFI1, partial SFI1 and no SFI1 signals.

Based on this, we do not believe that there is a correlation between SFI1 depletion efficiency and the strength of the structural defects, even if we cannot fully neglect this hypothesis. Instead, guided by the comment of Reviewer 2, we have now examined whether the structural defects arise early during centriole assembly or later. We captured top-viewed growing procentrioles and quantified the roundness index and general aspect (open / broken procentrioles). We found no difference between control and SFI1-depleted procentrioles, suggesting that the structural defects that we observed do not occur during centriole assembly. This new result indicates that it is rather the overall stability of mature centrioles that is altered upon SFI1 depletion. These new data are now reported in Figure 4 K, L and described in p. 11-12 of the revised manuscript. This result now might explain why the percentage of structurally abnormal centrioles is about 30% and not more penetrant- perhaps the stability of the mature centriole needs to be challenged over the course of the cell cycle to drive a more drastic phenotype. Another explanation could be that there are more defects but that the resolution limits relative to U-ExM prevents our visualizing them.

5. The authors state in Fig. S2B, C, F and H that a Sfi1 signal appeared in a more proximal region of the centriole and that this signal is decreasing after Sfi1 depletion. Why did the authors not investigate this second SFI1 pool?
We did not follow this faint localization as it was not always apparent, nor was it consistently located at a specific point. However, we felt that this observation still needs to be reported for the sake of the community, as we saw that the signal was reduced upon siRNA.

Reviewer #3 (Significance (Required)):

The manuscript determines convincingly the localisation of SFI1, centrin 2 and 3 and clearly shows how centrin is recruited to distinct sites within centrioles by POC5 and SFI1. However, because of the lack of functional data, the overall impact of the manuscript is limited.

Following the reviewer's comment as well as the proposed experiments from the two other reviewers, we significantly improved the manuscript by providing numerous additional experiments that greatly increase the amount of functional data. We have now resolved the apparent discrepancy between our study and the previous study by Kodani et al. and specifically strengthened our results by showing reproducibility in 3 different cell lines and rescue of all reported phenotypes, also using knockout cells in addition to siRNA as an further genetic model to bolster our findings.
Importantly, we now provide a molecular understanding of the ciliogenesis defects observed upon SFI1 depletion. Indeed, we found that CP110 decapping is defective in SFI1-depleted RPE-1 cells and that the distribution of the distal appendage protein, CEP164, is altered. This new result is fully consistent with the reported roles of Centrin-2 and strengthens the proposition that SFI1 and Centrin act as a complex at the centriole distal end. We hope that with these additions, the reviewer will be convinced that our study is now of significance to a broad community.

1st Editorial Decision 21st Jul 2022

Thank you for submitting your revised Review Commons manuscript for consideration by The EMBO Journal. In light of the constructive original comments and the considerable extensions over the original preprint, I decided to treat it similar to a regular revision, and sent it directly to two of the original referees for an assessment of your responses. Given their unanimous positive feedback (copied below for your information), we shall be happy to offer publication of this work in The EMBO Journal. Before we can formally accept the study, I would only need you to adjust the manuscript according to our formatting guidelines, and to address several editorial points, as follows:

Referee #1:

This is a revised version of an earlier manuscript submitted to "Review Commons". This revised manuscript contains several key experiments that make it now attractive to a broad readership.
I have read the new manuscript, as well as the authors' responses to the criticism raised in the previous review process. I feel that every important point has been carefully addressed experimentally and by re-writing the text.
It is now convincingly shown that SFI1 is a centriolar protein at the distal end that is responsible for the localization of a distal pool of centrins 2 and 3. It is further shown that loss of SFI1 leads to defects in centriolar architecture, but does not prevent duplication of centrioles, in contrast to previously published statements by others. Most importantly, the revised manuscript shows that SFI1 is necessary for correct localization of the distal appendage protein Cep164 and for the de-capping of CP110, to enable ciliogenesis.

Overall, this is a well-controlled study with excellent microscopy images that provides novel information on the roles of SFI1 in centriole assembly and ciliogenesis.

Referee #2:

In this revised manuscript, the authors responded to most of my comments by presenting the data from additional experiments as much as they could feasibly do. In the original manuscript, all reviewers pointed out that the data were relatively descriptive, which was a weak point of this study. However, even with respect to that, the authors have added new data that would give a mechanistic view of how SFI1 functions in centriole formation, especially with respect to the phenotype of ciliogenesis. For these reasons, I would consider this paper suitable for publication in EMBO J.

Referee #1:

This is a revised version of an earlier manuscript submitted to "Review Commons". This revised manuscript contains several key experiments that make it now attractive to a broad readership.
I have read the new manuscript, as well as the authors' responses to the criticism raised in the previous review process. I feel that every important point has been carefully addressed experimentally and by re-writing the text.
It is now convincingly shown that SFI1 is a centriolar protein at the distal end that is responsible for the localization of a distal pool of centrins 2 and 3. It is further shown that loss of SFI1 leads to defects in centriolar architecture, but does not prevent duplication of centrioles, in contrast to previously published statements by others. Most importantly, the revised manuscript shows that SFI1 is necessary for correct localization of the distal appendage protein Cep164 and for the de-capping of CP110, to enable ciliogenesis.

Overall, this is a well-controlled study with excellent microscopy images that provides novel information on the roles of SFI1 in centriole assembly and ciliogenesis.

We thank the reviewer for his/her positive feedback on our revised manuscript and are happy to read that he/she finds the data convincing.

Referee #2:

In this revised manuscript, the authors responded to most of my comments by presenting the data from additional experiments as much as they could feasibly do. In the original manuscript, all reviewers pointed out that the data were relatively descriptive, which was a weak point of this study. However, even with respect to that, the authors have added new data that would give a mechanistic view of how SFI1 functions in centriole formation, especially with respect to the phenotype of ciliogenesis. For these reasons, I would consider this paper suitable for publication in EMBO J.

We thank the reviewer for his/her positive feedback on our revised manuscript and his/her support for publication in EMBO J.

1st Revision - Editorial Decision 2nd Sep 2022

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

EMBO Press Author Checklist

Corresponding Author Name: Paoletti, Guichard, Hamel
Journal Submitted to: EMBO journal
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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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
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- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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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.
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
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  - are tests one-sided or two-sided?
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Select "Not Applicable" only when the requested information is not relevant for your study.

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For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

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Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
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If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	