THE EMBO JOURNAL

Spatial and proteomic profiling reveals centrosome-independent features of centriolar satellites

Ladan Gheiratmand, Etienne Coyaud, Gagan D. Gupta, Estelle M.N. Laurent, Monica Hasegan, Suzanna L. Prosser, João Gonçalves, Brian Raught and Laurence Pelletier

Review timeline:

Submission date:	10th Nov 2018
Editorial Decision:	13th Dec 2018
Revision received:	7th Apr 2019
Editorial Decision:	29th Apr 2019
Revision received:	9th May 2019
Accepted:	10th May 2019

Editor: Hartmut Vodermaier

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 13th Dec 2018

Thank you again for submitting your manuscript on centriolar satellite spatial and proteomic profiling to The EMBO Journal. It has now been seen by two expert referees, in light of whose positive feedback we shall be happy to consider this work further for publication, pending adequate revision of a number of specific queries raised in their reports. Since it is our policy to allow only a single round of experimental revision, please diligently respond to all points raised at this stage. We generally ` three months as standard revision time, and it is our policy that competing manuscripts published during this period will have no negative impact on our final assessment of your revised study.

REFEREE REPORTS:

Referee #1:

Centriolar satellites are multi-protein complexes that localizes near centrosomes and basal bodies of cilia. They move along microtubules by motor proteins and have been proposed to mediate the transport, exchange, or restriction of proteins between the centrosome and the cytoplasm to support the biogenesis and function of centrosomes and cilia. Although the function of individual centriolar satellite proteins has been investigated in recent years, the composition, function, and basic features of centriolar satellites remain incompletely defined.

The manuscript by Gheiratmand et al., provides a significant contribution to our understanding of centriolar satellites. They present a systematic proteomics analysis of these structures by using proximity mapping based on 22 of 44 known human satellite proteins. For the analyses, they establish stable cell lines for inducible expression of bait proteins with a FLAG-BirA* tag and convincingly confirm co-localization to PCM1 and in vivo biotinylation by streptavidin fluorescent labelling for all bait proteins. The proximity interaction analyses resulted in a network of 660 proteins enriched in known centrosome and satellite proteins clustered into core and peripheral

centriolar satellite proteins as well as sub-clusters indicative of novel functions associated with centriolar satellites.

Among the identified components, they tested 11 core or peripheral candidates and validated 6 as novel centriolar satellite proteins (TPGS1, CCDC138, CCDC22, CCDC92, CEP55, and CCDC57). TPGS1 is a subunit of the tubulin polyglutamylase complex and was further validated by reciprocal immunoprecipitation experiments supporting LRRC49 and C11orf49 as likely subunits of the human tubulin polyglutamylase complex. This suggests a potential role of centriolar satellites in the recruitment of tubulin polyglutamylation complexes and is supported by previously observed members of the tyrosine tubulin ligase-like family of enzymes (TTLL1 and TTLL5) that further modify glutamylated sites. This is an interesting finding for future investigation on how centriolar satellite via tubulin modifications might modulate microtubule-mediated transport in the vicinity of centrosomes or cilia. Beyond the validated proteins, the data provide a rich source of additional candidates and how they interact with core and peripheral centriolar satellite components. A subset of these candidates, such as potential plus-end directed kinesin motor proteins, is subject to further discussions in the manuscript.

A key aspect of the manuscript is the attention to fundamental features of centriolar satellites. The interaction network analysis is supportive of the idea that different populations of centriolar satellite are present in the cell. This is further evaluated by high resolution microscopy, which shows correlation between the MS-derived proximity data (merged with previously reported centrosome proximity data) and the co-localization distribution between PCM1 and representative centriolar satellite proteins as determined by high resolution microscopy. Importantly, they demonstrate that centriolar satellites remain associated in discrete assemblies when dispersed in the absence of centrioles or upon microtubule disassembly and conclude that centriolar satellites can assemble independently of centrosomes and microtubules. To what extent dispersed centriolar satellites remain functionally active entities remains to be further investigated.

Specific comments/suggestions

1. The manuscript is well written and convincingly supported by figures and experimental data.

2. For the initial bait protein selection, it is mentioned that proteins were excluded if they had been reported to display additional localizations. This argument make sense in terms of determining the core centriolar satellite protein composition but appears less logical if the aim is to expand the functional landscape of centriolar satellites. According to the Human Protein Atlas, most proteins are multi-localizing.

3. The proximity-labelling strategy enable interaction analysis under native conditions in living cells and adaptation to the tagged protein are prevented here by inducible expression. Since overexpression of centriolar satellite proteins might perturb the subcellular distribution of satellites, as mentioned for Cep290, it would be useful with a comment about bait protein expression levels and the potential impact on satellite redistribution, which in itself constitute useful information. Some differences are observed for PCM1 in Figure EV1 but are these differences significant and are this considered for the interpretation of the proximity-based interaction network?

4. How is the data normalized in between proximity labelling experiments?

5. Some localization data has previously been reported for CCDC92 (PMID 22863007).

6. The proximity data are displayed as protein-protein interaction networks derived from statistical analysis using the SAINTexpress software. Quantitation is based on spectral counting, which is a semi-quantitative measure of protein abundance in label-free proteomic studies. Provided the effort invested in establishing the experimental system, it is surprising that the analysis do not take into account the intensity values of the identified peptides for a more accurate quantitation. Network topology also appears to be based on counting the number of baits interacting with each prey. Although statistical power is gained from a large number of experiments, it could be interesting with a few plots that directly visualize the enrichment of proximity-labelled proteins using spectral counting and peptide intensities. This might also serve to indicate to what extent single or multiple bait experiments are recommended for proximity labelling experiments.

7. The presented data on satellites associated with or dispersed from centrosomes, provides a unique opportunity to resolve proteins shared (or not) between satellites and centrosomes/cilia. Although a few examples are discussed (e.g. Cep55), it could be interesting with a closer look at this aspect and if some general trends can be observed. Are all centrosomal proteins companions of centriolar satellites to some degree?

8. The study contributes detailed insight into the composition, function, and basic features of centriolar satellites. Could this help to better define these subcellular structures?

Referee #2:

Centriolar satellites are cytoplasmic particles found in vertebrate cells characterized by the presence of the scaffolding component PCM1. Localized in a dynamic manner in the vicinity of the centrosome and carrying a diverse cargo of centriolar, centrosomal and ciliary components, satellites have been linked to the assembly and function of centrosomes as well as cilia. Despite a large body of work spanning two decades, the functional significance of centriolar satellites remains poorly understood. Here, Gheiratmand and colleagues undertook a proteomic approach to delineate the protein composition of satellites by BioID-based proximity mapping. In principle this has been a worthwhile endeavor and the resulting data should be of interest to the readership of the EMBO Journal. However, as detailed below there are several critical issues that need to be addressed prior to publication.

To briefly summarize the main data presented in this manuscript, the authors build on a previous proximity mapping study (Gupta et al, Cell 2015), extending their coverage of centriolar satellite baits from 5 to 22. With this larger dataset in hand the authors create an interaction map revealing an inner core of known and suspected satellite components as well as a more peripheral set of interactors. They further confirm the satellite localization of 6 candidate proteins, validating their proteomics approach. Finally, they report that satellite composition is unchanged by loss of centrosomes, here induced by centrinone-induced PLK4 inhibition.

Main points
1. As the authors also state in the text, this manuscript is an extension of their prior work on proximity mapping of centrosomal and ciliary proteins. What is somewhat unclear is whether they have repeated their proximity mapping for the 5 centriolar satellites previously analyzed by Gupta et al, or if those results were carried over into this work. If the latter, this should be clearly stated. If the former, a comparison of the BioID hits for each of these 5 baits should be presented as in Fig EV1B.

2. There is a striking difference in length of the original list of potential interactions revealed by mass spectrometry (38451 raw hits) and the filtered list of high confidence interactions presented in Fig 1A (2113 interactions representing 660 unique interactors). Clearly, the cutoff BFDR {less than or equal to} 0.01 is key. The authors then use prey-centered correlation to identify and subsequently confirm TPGS1, which does not nearly reach the significance threshold, as a further satellite component (Fig 3). How confident are they, then, that a protein like gamma-tubulin (TUBG1), present in multiple BioID datasets, is not also a satellite component?

3. In Fig 5, the authors report that centriolar satellite composition does not change upon centrinone-induced centriole depletion. This finding currently rests on a rather weak foundation, as (somewhat surprisingly after 7 days of inhibitor treatment) 40% of cells still retain their centrioles (Fig 5B). With these cells likely contributing more or less wild-type satellites to the extracts used for BioID, the apparent persistence of the satellite interaction network may simply reflect that high background.

4. One of the authors' main points in this study is that different centriolar satellite populations exist. This is primarily based on network analysis of the interactions identified by BioID. However, in Fig 4 they link this to the degree of colocalization between PCM1 and various other satellite components, ranging from near-complete colocalization (OFD1) to nearly none (PLK1S1). There are two ways to interpret this: One is that there are satellites not containing PCM1. The other is that the majority of certain proteins is on other cellular structures, most probably the centrosome. This

should be readily apparent from the imaging data and should be clarified as such.

5. Related to the previous point, one key experiment missing from the study is the impact of PCM1 depletion on the satellite network established by the authors. If PCM1 is truly essential for scaffolding all satellites, the entire network reported by the authors should collapse. Alternatively, the authors may find that satellites have an existence independent of PCM1. While the authors may consider this beyond the scope of the current study, this is perhaps the most important outstanding experiment and essential for a proper understanding of satellite composition.

Other points
6. Besides being colorful, what is the rationale for assigning proteins to different categories in Fig 1C, D? This seems rather arbitrary. I would argue, for example, that both C2CD3 and CEP135 are centriolar proteins, not satellite or 'centrosome' (ie PCM) components as suggested by the authors.

7. Fig 6C seeks to illustrate that specfic centriolar satellite components still colocalize following nocodazole and centrinone treatment. Without presenting each channel individually, this is impossible to tell from the merge.

8. It is disingenuous to state that the effect of disruption of the MT network on satellite composition was not previously assessed (p18). The paper referred to by the authors (Dammermann & Merdes, 2002) showed exactly what they now confirm: the association of PCM1 with other satellite components remains unchanged (see their Fig 6).

9. While I agree with the authors that the precise composition and assembly properties of satellites remains a 'mystery' (abstract), this is perhaps not the best term to use in a scientific manuscript. Similarly, 'hairball' may be an apt term to describe the network in Fig 1C (p38), but really?

10. The introduction is somewhat misleading in that depletion of PCM1 does not restrict proteins like NIN, CETN2 and PCNT to centrosomes or result in cytoplasmic aggregates (p4/5). No aggregates were reported and centrosomal accumulation was reduced, consistent with the model of PCM1 as a satellite scaffolding factor essential for assembly of centrosomal cargo.

11. The use of 'CS' for centriolar satellites throughout the manuscript seems unnecessarily confusing (this is a term I would rather associate with centrosomes, not satellites). 'Satellites' would hardly be longer and certainly less ambiguous.

Concluding thoughts

12. A question that remains unanswered in this manuscript is if so many centrsomal and ciliary components are found on satellites, are we just talking about a mobile reservoir of proteins surplus to requirements or as the authors aparently believe a unique cellular compartment with potentially independent cellular functions? In other words is it just (somewhat ironically) the absence of centrioles that defines satellites, or are there centrosomal components clearly NOT present at satellites? In a manuscript of this type, this is something I would expect the authors to speculate on.

1st Revision - authors' response 7th Apr 2019

We would like to thank the referees for their enthusiasm about our work, judicious comments and the many thoughtful suggestions. Below is our detailed point-by point response (our responses are in bold and the original comments in their entirety are in *italics*). The referee reports have been very helpful and we hope that the reviewers will now find the revised version of our manuscript suitable for publication in *EMBO J.*

Referee #1:

Centriolar satellites are multi-protein complexes that localizes near centrosomes and basal bodies of cilia. They move along microtubules by motor proteins and have been proposed to mediate the

transport, exchange, or restriction of proteins between the centrosome and the cytoplasm to support the biogenesis and function of centrosomes and cilia. Although the function of individual centriolar satellite proteins has been investigated in recent years, the composition, function, and basic features of centriolar satellites remain incompletely defined.

The manuscript by Gheiratmand et al., provides a significant contribution to our understanding of centriolar satellites. They present a systematic proteomics analysis of these structures by using proximity mapping based on 22 of 44 known human satellite proteins. For the analyses, they establish stable cell lines for inducible expression of bait proteins with a FLAG-BirA tag and convincingly confirm co-localization to PCM1 and in vivo biotinylation by streptavidin fluorescent labelling for all bait proteins. The proximity interaction analyses resulted in a network of 660 proteins enriched in known centrosome and satellite proteins clustered into core and peripheral centriolar satellite proteins as well as sub-clusters indicative of novel functions associated with centriolar satellites.*

Among the identified components, they tested 11 core or peripheral candidates and validated 6 as novel centriolar satellite proteins (TPGS1, CCDC138, CCDC22, CCDC92, CEP55, and CCDC57). TPGS1 is a subunit of the tubulin polyglutamylase complex and was further validated by reciprocal immunoprecipitation experiments supporting LRRC49 and C11orf49 as likely subunits of the human tubulin polyglutamylase complex. This suggests a potential role of centriolar satellites in the recruitment of tubulin polyglutamylation complexes and is supported by previously observed members of the tyrosine tubulin ligase-like family of enzymes (TTLL1 and TTLL5) that further modify glutamylated sites. This is an interesting finding for future investigation on how centriolar satellite via tubulin modifications might modulate microtubule-mediated transport in the vicinity of centrosomes or cilia. Beyond the validated proteins, the data provide a rich source of additional candidates and how they interact with core and peripheral centriolar satellite components. A subset of these candidates, such as potential plus-end directed kinesin motor proteins, is subject to further discussions in the manuscript.

An key aspect of the manuscript is the attention to fundamental features of centriolar satellites. The interaction network analysis is supportive of the idea that different populations of centriolar satellite are present in the cell. This is further evaluated by high resolution microscopy, which shows correlation between the MS-derived proximity data (merged with previously reported centrosome proximity data) and the co-localization distribution between PCM1 and representative centriolar satellite proteins as determined by high resolution microscopy. Importantly, they demonstrate that centriolar satellites remain associated in discrete assemblies when dispersed in the absence of centrioles or upon microtubule disassembly and conclude that centriolar satellites can assemble independently of centrosomes and microtubules. To what extent dispersed centriolar satellites remain functionally active entities remains to be further investigated.

Specific comments/suggestions:

1. The manuscript is well written and convincingly supported by figures and experimental data.

We thank the reviewer for the positive feedback, it is very much appreciated!

2. For the initial bait protein selection, it is mentioned that proteins were excluded if they had been reported to display additional localizations. This argument make sense in terms of determining the core centriolar satellite protein composition but appears less logical if the aim is to expand the functional landscape of centriolar satellites. According to the Human Protein Atlas, most proteins are multi-localizing.

We have attempted to clarify this issue on Page 8 of the revised manuscript. While it is certainly the case that many proteins localize to multiple intracellular locations, in some cases the Human Protein Atlas data does not agree with our own observations and must be viewed with some caution. For example, the Human Protein Atlas indicates that PCM1 localizes to the centrosome, nuclear membrane and cytosol, and that WRAP73 localizes to the nucleoplasm and cell junctions. In our hands PCM1 and WRAP73 both localize primarily to satellites and the vicinity of centrosomes. Our BioID results back up these findings. We do agree that our

initial statement was poorly worded, however, and now simply states that we tagged 22 proteins known to display "prominent" satellite localization patterns.

We would also like to emphasize that one of the strengths of BioID is the ability to identify transient interactions that are not likely to be captured using more standard (e.g. IP-based) techniques. Indeed, this approach allowed us to uncover both a "core" centriolar satellite network as well as many other potential "peripheral" interactions (highlighted in Fig 1C) and has in fact significantly expanded the functional landscape of centriolar satellites. Conducting BioID on the rest of the satellite proteins will give us a much clearer idea about their overall function and further delineate their association with other organelles. This is now mentioned in the discussion on Page 24.

3. The proximity-labelling strategy enable interaction analysis under native conditions in living cells and adaptation to the tagged protein are prevented here by inducible expression. Since overexpression of centriolar satellite proteins might perturb the subcellular distribution of satellites, as mentioned for Cep290, it would be useful with a comment about bait protein expression levels and the potential impact on satellite redistribution, which in itself constitute useful information. Some differences are observed for PCM1 in Figure EV1 but are these differences significant and are this considered for the interpretation of the proximity-based interaction network?

As shown in previous studies, in this system the FLAG-BirA* tagged proteins are generally expressed at close to endogenous levels (Liyanage, Coyaud et al., 2017, Odeh, Coyaud et al., 2018). In this particular case, centriolar satellite baits were induced with titrated levels of tetracycline (0.001- 1 μg/ml) to avoid overexpression artifacts. For example, we confirmed that inducing the expression of the tagged bait proteins for 24h does not affect satellite morphology based on PCM1 intensity and the total area satellites occupy. This is shown for three different baits (FLAG-BirA* CEP290, CCDC14 and CEP72) before and after induction with Tet (Appendix Fig S2). The localization of all bait proteins to centriolar satellites was also confirmed prior to performing BioID (Fig EV1A). This is now mentioned on Page 8 of the revised manuscript and detailed more precisely in the methods section on Page 32.

4. How is the data normalized in between proximity labelling experiments?

There is no normalization done (or required) for this type of data analysis. We do conduct standard weekly quality control procedures to ensure that our instruments are tuned and calibrated properly, and we analyze the same amount of material (~1ug total peptides) for each sample. The type of data analysis conducted in this manuscript (and all of our other BioID papers) (Comartin, Gupta et al., 2013, Coyaud, Ranadheera et al., 2018, Gupta, Coyaud et al., 2015, Yeh, Coyaud et al., 2015) uses Bayesian statistical approaches to assign a confidence value indicating whether a particular bait-prey interaction is *true* (*i.e.* sufficient evidence exists to reject the null hypothesis that the spectral counts observed for a specific bait-prey interaction are significantly different from those observed in the control runs) or *false* (the spectral counts do not differ significantly from those observed in 20 control runs, and it must therefore be considered "noise" in the system *e.g.* non-specific binding to the streptavidin-sepharose). The strength of this type of analysis is that it is independent of other considerations such as bait protein size, expression levels, and the many other variables inherent to this type of work (Teo, Liu et al., 2014). This is now mentioned in the methods section of the revised manuscript on Page 34.

5. Some localization data has previously been reported for CCDC92 (PMID 22863007).

We thank the reviewer for pointing this out and apologize for the omission. This finding is now mentioned in the text on Page 13 and referenced accordingly.

6. The proximity data are displayed as protein-protein interaction networks derived from statistical analysis using the SAINTexpress software. Quantitation is based on spectral counting, which is a semi-quantitative measure of protein abundance in label-free proteomic studies. Provided the effort invested in establishing the experimental system, it is surprising that the analysis do not take into account the intensity values of the identified peptides for a more accurate quantitation. Network topology also appears to be based on counting the number of baits interacting with each prey.

Although statistical power is gained from a large number of experiments, it could be interesting with a few plots that directly visualize the enrichment of proximity-labelled proteins using spectral counting and peptide intensities. This might also serve to indicate to what extent single or multiple bait experiments are recommended for proximity labelling experiments.

The reviewer is suggesting that we might want to use MS1 peptide intensities instead of spectral counting. However, it must be noted that MS1-based methods come with their own important caveats, such as – choice of automated background correction methods (*e.g.* sometimes this software can be somewhat arbitrary at predicting where to draw "background" noise cutoff lines), mis-assignment of MS1 peaks, extensive peak overlap with co-eluting species, signal suppression during busy elution times, etc. Due to these and other common issues with MS1 approaches, a large number of previous publications (starting from more than a decade ago) have argued that there is actually little or no added value to using MS1 values versus peptide counts (Liu, Sadygov et al., 2004) 76:4193; (Colinge, Chiappe et al., 2005) 77:596; (Old, Meyer-Arendt et al., 2005) 4:1487; (Zybailov, Coleman et al., 2005)). MS1 approaches should thus not be considered to be somehow more "accurate" or quantitative than spectral counting: the CVs between the two types of methods are actually quite similar, yet spectral counting is much simpler, requires much less data manipulation, and much less computational resources and time. If the editor and/or reviewers prefer that we address this point in the main text, we would be happy to do so but have left this out for now.

Network topology also appears to be based on counting the number of baits interacting with each prey. Although statistical power is gained from a large number of experiments, it could be interesting with a few plots that directly visualize the enrichment of proximity-labelled proteins using spectral counting and peptide intensities. This might also serve to indicate to what extent single or multiple bait experiments are recommended for proximity labelling experiments.

The map topology in this manuscript is based on both peptide counts and the number of baits interacting with a given prey (specifically we generally use a "Prefuse Force directed layout", with spectral counts as the force parameter, and additional parameters as described in Materials and Methods).

However, to address the reviewer's question, we now include the same analysis conducted without *any* quantitative information (Appendix Fig S3). As you can see, the topology is almost exactly the same, indicating that any type of quantitation – including use of the suggested MS1 values - would have little or no effect on the topology of this tightly clustered map. This is likely due, as the reviewer suggests, to the high number of bait interactions for the prey proteins in the "core" region of the map.

The reviewer raises an interesting question in understanding how many baits might be required to map a given complex or intracellular structure. We would argue that this is essentially unknowable *a priori* because there are just so many variables involved this type of analysis. However, we mention in the manuscript (Page 15) that at some point as more baits were added to the satellite map, no additional prey proteins were detected – suggesting that we had reached saturation of the structure (at least as far as BioID is concerned). If this analysis were conducted in an iterative manner, I suppose it could be argued that we would be justified in stopping once this saturation level was reached.

7. The presented data on satellites associated with or dispersed from centrosomes, provides a unique opportunity to resolve proteins shared (or not) between satellites and centrosomes/cilia. Although a few examples are discussed (e.g. Cep55), it could be interesting with a closer look at this aspect and if some general trends can be observed. Are all centrosomal proteins companions of centriolar satellites to some degree?

This is a great suggestion, thank you. We now include a comparison of the BioID data from our earlier paper (Gupta et al.), consisting of the interactome of 31 centrosome baits versus the centriolar satellite baits analyzed here (Appendix Fig S7, Appendix Table S2 – ciliary transition zone baits were not included in this comparison). Overall, 34.3% of the 30 bait centrosomal interactome (Gupta) was also detected in the satellite bait interactome. Conversely, 39.9% of the satellite interactome overlaps with the centrosome dataset. Thus,

while significant overlap is observed between the two interactomes, the majority of each dataset is specific to the centrosome or satellite. To make this point more clearly, we have also highlighted several examples of "organelle-specific" prey proteins that are detected by 4-5 bait proteins uniquely in either the centriole or satellite.

Interestingly, the satellite "core" proteins (i.e. those 84 prey polypeptides detected by 11 or more satellite bait proteins) did share significant overlap with the centrosome interactome, suggesting that these highly connected proteins could play important roles in both structures. We have added this statement to the manuscript on Page 29.

Furthermore, we also noted that the 20 centriolar and centriolar appendage baits present in our satellite interactome are not lost upon centriole depletion indicating that their localization to satellites is centrosome-independent. This is now discussed on Page 29 of the revised manuscript.

8. The study contributes detailed insight into the composition, function, and basic features of centriolar satellites. Could this help to better define these subcellular structures?

Yes, we think so, absolutely! Despite the existence of a number of studies on specific centriolar satellites, many questions remain to be answered. As this reviewer suggests, our study has shed light on several unknown aspects of centriolar satellite biogenesis and function including their composition, their potential role in tubulin modification and their mode of assembly. We believe that these findings could be useful for further studies in the field and would help in improving our understanding of the satellites and their structure. This is now emphasized in the revised discussion.

Referee #2:

Centriolar satellites are cytoplasmic particles found in vertebrate cells characterized by the presence of the scaffolding component PCM1. Localized in a dynamic manner in the vicinity of the centrosome and carrying a diverse cargo of centriolar, centrosomal and ciliary components, satellites have been linked to the assembly and function of centrosomes as well as cilia. Despite a large body of work spanning two decades, the functional significance of centriolar satellites remains poorly understood. Here, Gheiratmand and colleagues undertook a proteomic approach to delineate the protein composition of satellites by BioID-based proximity mapping. In principle this has been a worthwhile endeavor and the resulting data should be of interest to the readership of the EMBO Journal. However, as detailed below there are several critical issues that need to be addressed prior to publication.

To briefly summarize the main data presented in this manuscript, the authors build on a previous proximity mapping study (Gupta et al, Cell 2015), extending their coverage of centriolar satellite baits from 5 to 22. With this larger dataset in hand the authors create an interaction map revealing an inner core of known and suspected satellite components as well as a more peripheral set of interactors. They further confirm the satellite localization of 6 candidate proteins, validating their proteomics approach. Finally, they report that satellite composition is unchanged by loss of centrosomes, here induced by centrinone-induced PLK4 inhibition.

Main points:
1. As the authors also state in the text, this manuscript is an extension of their prior work on proximity mapping of centrosomal and ciliary proteins. What is somewhat unclear is whether they have repeated their proximity mapping for the 5 centriolar satellites previously analyzed by Gupta et al, or if those results were carried over into this work. If the latter, this should be clearly stated. If the former, a comparison of the BioID hits for each of these 5 baits should be presented as in Fig EV1B.

We are happy to clarify this and apologize for not doing so in the first instance. Our mass spectrometry infrastructure was significantly upgraded (from Velos orbitrap to QE-HF systems) since the publication of our original BioID study of the centrosome-cilium interface.

To be consistent, we repeated the BioID analysis of the 5 original centriolar satellite proteins previously described in Gupta *et al.*

The newer instruments are both significantly faster (i.e. generate more MS2 fragmentation events per second) and more sensitive, and thus provide us with deeper coverage of the BioID samples. Nevertheless, the interactors detected for a given bait in both instances is quite consistent. We have included a direct comparison of Velos (Gupta et al., 2015) vs QE-HF (This study) data in Appendix Fig S1. The R^2 value, based on spectral counts for all high confidence interactors reported here, averages 0.85 indicating that – even though these analyses were conducted on different instruments several years apart – the BioID-MS is quite reproducible. This is now mentioned on Page 8 of the revised manuscript.

2. There is a striking difference in length of the original list of potential interactions revealed by mass spectrometry (38451 raw hits) and the filtered list of high confidence interactions presented in Fig 1A (2113 interactions representing 660 unique interactors). Clearly, the cutoff BFDR {less than or equal to} 0.01 is key. The authors then use prey-centered correlation to identify and subsequently confirm TPGS1, which does not nearly reach the significance threshold, as a further satellite component (Fig 3). How confident are they, then, that a protein like gamma-tubulin (TUBG1), present in multiple BioID datasets, is not also a satellite component?

Indeed, the dramatic filtering (38000 to 2100 hits) is quite standard for this type of analysis, and reflects the stringency achieved using the analytical tools, and low FDR, that we use here.

It is true that we did not observe TPGS1 to be a high confidence interactor with any of the 22 satellite bait proteins tested here. As explained in the original manuscript, TPGS1 clustering with known satellite components was observed in the context of a large *merged* dataset consisting of our previous centrosome/cilia interactome and the current satellite dataset. Consistent with previous reports (Regnard, Fesquet et al., 2003), using BioID we picked up TPGS1 as an interactor with several different centrosome proteins (e.g. STIL, CEP135 and CEP290) in the Gupta et al. paper and here in Flag-tagged centriolar satellite IPs (in this complementary approach, the control spectral counts were all 0) as shown in Fig 3G. Prey-prey clustering is an interesting and powerful approach that allows us to mine interactome datasets by simply calculating how often a given protein is observed *along with* any other prey protein in the dataset. In this case, TPGS1 was observed as a high confidence interactor with bait proteins that also interacted with C11orf49 and LRRC49. This result suggested that TPGS1 could be working with C11orf49 and LRRC49 in a functional module and our follow up experiments – using both colocalization with PCM1, and BioID of the TPGS1 protein itself - confirmed that TPGS1 is a *bona fide* satellite component (Fig 3).

The general point made by the reviewer is of course an important one, and a known caveat of this type of approach to identify bait-prey relationships. That is, our analysis is completely dependent on the use of extensive negative controls, allowing us to differentiate between specific and non-specific interactors of a given bait protein, in a given cell type. It is certainly true that we could be missing some interacting partners e.g. in cases where a prey protein also displays strong binding to the streptavidin-sepharose solid phase support. If the difference in spectral counts (between control and experimental) is not deemed to be sufficiently large enough by the SAINT algorithm, then these identifications will be missed. These types of false-negatives are a known limitation of the system.

3. In Fig 5, the authors report that centriolar satellite composition does not change upon centrinone-induced centriole depletion. This finding currently rests on a rather weak foundation, as (somewhat surprisingly after 7 days of inhibitor treatment) 40% of cells still retain their centrioles (Fig 5B). With these cells likely contributing more or less wild-type satellites to the extracts used for BioID, the apparent persistence of the satellite interaction network may simply reflect that high background.

Like this reviewer, we were surprised at the inefficient depletion of centrioles in HEK293 cells. During the course of our study we attempted to increase the efficiency of centriole depletion in this cell line by titrating the centrinone concentration and treatment duration. For example, we tried multiple doses (350, 500, 750, 1000 and 1500 nM) for up to 12 days, and yet

consistently observed that about 40% of the cells retained their centrioles. We do not know the reason for this but can speculate that for some reason, PLK4-independent initiation of centriole duplication and/or differential regulation of PLK4 activity occurs in this cell background.

To confirm that satellites are still present, and that their interactions are maintained in the context of a more efficient centriole depletion, we used a U-2 OS cell line in which the centriole duplication factor STIL was disrupted using CRISPR/Cas9 (see Appendix Fig S6A) (Liu, Gupta et al., 2018). Staining for PCM1 and the other satellite markers revealed that centriolar satellite structures are still present in STIL KO cells but that they are now dispersed throughout the cell, with no accumulation in the perinuclear region (Appendix Fig S6B). To determine if satellite protein interactions are altered in the absence of centrioles, we performed co-IP in STIL KO and WT U-2 OS cells transiently expressing FLAG-BirA* or FLAG-BirA*-PCM1 (Appendix Fig S6C). Interactions between PCM1 and the satellite proteins tested (CEP131, OFD1 and CEP72) were still detected in the absence of centrioles (Appendix Fig S6D). Together, these observations are consistent with our MS data, suggesting that the satellite proximity interaction landscape is largely unaffected in the absence of centrioles. These data are now added to page 20-21 of the revised manuscript.

4. One of the authors' main points in this study is that different centriolar satellite populations exist. This is primarily based on network analysis of the interactions identified by BioID. However, in Fig 4 they link this to the degree of colocalization between PCM1 and various other satellite components, ranging from near-complete colocalization (OFD1) to nearly none (PLK1S1). There are two ways to interpret this: One is that there are satellites not containing PCM1. The other is that the majority of certain proteins is on other cellular structures, most probably the centrosome. This should be readily apparent from the imaging data and should be clarified as such.

This is a good point. Based on the growing number of satellite constituents, their wide range of activities, and our clustering and correlation data (Fig 4), individual satellites are likely to be composed of different subgroups of proteins to serve different functions. This has been alluded to previously (Kodani, Yu et al., 2015), but the proteomics and spatial satellite groups we have defined here are the first direct demonstration of this. Based on varying levels of colocalization between PCM1 and other satellite proteins, we can speculate that PCM1 is present only on a subset of centriolar satellite structures. Another possibility could be that PCM1 associates more transiently with some populations of centriolar satellites, which leads to a steady-state distribution predominantly to specific sub-populations. We also find that some satellite proteins also localize to centrosomes and other subcellular structures, which highlights the potential interplay between distinct centriolar satellite populations and other cellular functions. Systematic functional profiling of the various satellite populations in diverse cellular readouts will allow this hypothesis to be thoroughly tested in the future.
This matter is now included in the discussion on Page 27-28.

5. Related to the previous point, one key experiment missing from the study is the impact of PCM1 depletion on the satellite network established by the authors. If PCM1 is truly essential for scaffolding all satellites, the entire network reported by the authors should collapse. Alternatively, the authors may find that satellites have an existence independent of PCM1. While the authors may consider this beyond the scope of the current study, this is perhaps the most important outstanding experiment and essential for a proper understanding of satellite composition.

We appreciate this suggestion. To investigate whether PCM1 disruption alters the satellite proximity interaction landscape, we used the CRISPR/Cas9 strategy to disrupt the *PCM1* gene in four HEK293 inducible cell lines (FLAG-BirA* tagged CCDC66, CCDC14, PIBF1 and TEX9) and the FLAG-BirA* alone (see methods). PCM1 depletion was confirmed by western blotting and IF (Figs 5A-B and EV4B-C). In contrast to our results with RPE-1 cells, we were not able to fully knock out PCM1 in HEK293 cells. These cell lines did however display a significant decrease in PCM1 expression levels, (estimated ~10% or less of WT levels; Fig 5A and Fig EV4B. IF analyses in HEK293 or RPE-1 PCM1 knockout lines indicated that PCM1 localization to satellite-like structures is dramatically reduced with a restricted localization of OFD1, CEP72, CEP131, PIBF1 and CEP290, and biotinylated proteins to the immediate vicinity of the centrosomes (Figs 5B and EV4A-C). In line with our WB results, and in contrast

with the RPE-1 PCM1 KO, a fraction of HEK293 cells with seemingly intact satellites could be observed, likely due to cell to cell heterogeneity in the level of PCM1 KO in this cell pool.

To investigate how PCM1 loss affects the satellite proximity interaction landscape, we performed BioID on four bait proteins expressed in PCM1 KO cell lines and isogeneic WT HEK293 cells (Table EV6). Amongst the 84 core satellite proteins defined by the 22 baits in WT HEK293 cells (Fig 1C), 82 were detected by at least one of the four baits characterized in PCM1 KO HEK293 cells. However, consistent with satellite disruption in PCM1 KO cells, bait-prey clustering highlighted a number of lost bait-prey interactions (Fig 5E, orange and red boxes). For example, CEP72 and C2CD3 are lost as preys in all four baits upon PCM1 depletion. Other satellite preys such as CCDC18, PIBF1 or CCDC14 were lost by at least two baits. Interestingly, however, while the satellite structures are missing in most cells, many of the underlying protein-protein interactions were maintained (Fig 5E, white boxes). Several different subgroups of proteins that are either known to function together (e.g. Group1, CCP110 and CEP97) or which display similarities in their interaction patterns (i.e. share a similar pattern of prey protein losses in PCM1 KO cells) were manually annotated. These groups are likely to represent functional protein modules, since they maintain proximity interactions even in the absence of intact centriolar satellites (Fig 5E, circled numbers and 5F). This is now discussed on Pages 16-18 of the revised manuscript.

Other points:
6. Besides being colorful, what is the rationale for assigning proteins to different categories in Fig 1C, D? This seems rather arbitrary. I would argue, for example, that both C2CD3 and CEP135 are centriolar proteins, not satellite or 'centrosome' (ie PCM) components as suggested by the authors.

We are happy to clarify this issue. The color coding of the prey proteins is hierarchical starting from the satellites to centrosomes and below (yellow to grey) meaning that if a protein is known as both satellite and centrosome component, it is colored for satellite. For simplicity we also grouped centriolar proteins and PCM proteins into a generic "centrosome" category to facilitate visualization. This has been clarified in the legend of Fig 1C.

7. Fig 6C seeks to illustrate that specific centriolar satellite components still colocalize following nocodazole and centrinone treatment. Without presenting each channel individually, this is impossible to tell from the merge.

Individual channels have been added as Fig EV6D.

8. It is disingenuous to state that the effect of disruption of the MT network on satellite composition was not previously assessed (p18). The paper referred to by the authors (Dammermann & Merdes, 2002) showed exactly what they now confirm: the association of PCM1 with other satellite components remains unchanged (see their Fig 6).

We apologize for this misleading statement which has been deleted. This was not our intention. We wanted to emphasize that changes on the global proximity interaction landscape of centriolar satellites had not been investigated.

9. While I agree with the authors that the precise composition and assembly properties of satellites remains a 'mystery' (abstract), this is perhaps not the best term to use in a scientific manuscript. Similarly, 'hairball' may be an apt term to describe the network in Fig 1C (p38), but really?

Thank you for these comments. We have changed our wording to 'poorly explored' and 'network' respectively.

10. The introduction is somewhat misleading in that depletion of PCM1 does not restrict proteins like NIN, CETN2 and PCNT to centrosomes or result in cytoplasmic aggregates (p4/5). No aggregates were reported and centrosomal accumulation was reduced, consistent with the model of PCM1 as a satellite scaffolding factor essential for assembly of centrosomal cargo.

This sentence has been replaced with "in the absence of PCM1, the level of certain centrosome components including NIN, CETN2 and PCNT are markedly reduced".

11. The use of 'CS' for centriolar satellites throughout the manuscript seems unnecessarily confusing (this is a term I would rather associate with centrosomes, not satellites). 'Satellites' would hardly be longer and certainly less ambiguous.

The abbreviated form 'CS' has been replaced with centriolar satellites, or satellite(s), throughout the manuscript, tables, legends and figures.

Concluding thoughts

12. A question that remains unanswered in this manuscript is if so many centrosomal and ciliary components are found on satellites, are we just talking about a mobile reservoir of proteins surplus to requirements or as the authors apparently believe a unique cellular compartment with potentially independent cellular functions? In other words is it just (somewhat ironically) the absence of centrioles that defines satellites, or are there centrosomal components clearly NOT present at satellites? In a manuscript of this type, this is something I would expect the authors to speculate on.

We agree with the reviewer that this is an important point that was not adequately addressed in the original manuscript. A brief discussion on this topic is included on Page 29 of the revised manuscript.

References

Colinge J, Chiappe D, Lagache S, Moniatte M, Bougueleret L (2005) Differential proteomics via probabilistic peptide identification scores. *Anal Chem* 77: 596-606
Comartin D, Gupta GD, Fussner E, Coyaud E, Hasegan M, Archinti M, Cheung SW, Pinchev D, Lawo S, Raught B, Bazett-Jones DP, Luders J, Pelletier L (2013) CEP120 and SPICE1 cooperate with CPAP in centriole elongation. *Curr Biol* 23: 1360-6
Coyaud E, Ranadheera C, Cheng D, Goncalves J, Dyakov BJA, Laurent EMN, St-Germain J, Pelletier L, Gingras AC, Brumell JH, Kim PK, Safronetz D, Raught B (2018) Global Interactomics Uncovers Extensive Organellar Targeting by Zika Virus. *Mol Cell Proteomics* 17: 2242-2255
Gupta GD, Coyaud E, Goncalves J, Mojarad BA, Liu Y, Wu Q, Gheiratmand L, Comartin D, Tkach JM, Cheung SW, Bashkurov M, Hasegan M, Knight JD, Lin ZY, Schueler M, Hildebrandt F, Moffat J, Gingras AC, Raught B, Pelletier L (2015) A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell* 163: 1484-99
Kodani A, Yu TW, Johnson JR, Jayaraman D, Johnson TL, Al-Gazali L, Sztriha L, Partlow JN, Kim H, Krup AL, Dammermann A, Krogan NJ, Walsh CA, Reiter JF (2015) Centriolar satellites assemble centrosomal microcephaly proteins to recruit CDK2 and promote centriole duplication. *Elife* 4
Liu H, Sadygov RG, Yates JR, 3rd (2004) A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal Chem* 76: 4193-201
Liu Y, Gupta GD, Barnabas DD, Agircan FG, Mehmood S, Wu D, Coyaud E, Johnson CM, McLaughlin SH, Andreeva A, Freund SMV, Robinson CV, Cheung SWT, Raught B, Pelletier L, van Breugel M (2018) Direct binding of CEP85 to STIL ensures robust PLK4 activation and efficient centriole assembly. *Nat Commun* 9: 1731
Liyanage SU, Coyaud E, Laurent EM, Hurren R, Maclean N, Wood SR, Kazak L, Shamas-Din A, Holt I, Raught B, Schimmer A (2017) Characterizing the mitochondrial DNA polymerase gamma interactome by BioID identifies Ruvbl2 localizes to the mitochondria. *Mitochondrion* 32: 31-35
Odeh HM, Coyaud E, Raught B, Matunis MJ (2018) The SUMO-specific isopeptidase SENP2 is targeted to intracellular membranes via a predicted N-terminal amphipathic alpha-helix. *Mol Biol Cell* 29: 1878-1890
Old WM, Meyer-Arendt K, Aveline-Wolf L, Pierce KG, Mendoza A, Sevinsky JR, Resing KA, Ahn NG (2005) Comparison of label-free methods for quantifying human proteins by shotgun proteomics. *Mol Cell Proteomics* 4: 1487-502
Regnard C, Fesquet D, Janke C, Boucher D, Desbruyeres E, Koulakoff A, Insina C, Travo P, Edde B (2003) Characterisation of PGs1, a subunit of a protein complex co-purifying with tubulin polyglutamylase. *J Cell Sci* 116: 4181-90
Teo G, Liu G, Zhang J, Nesvizhskii AI, Gingras AC, Choi H (2014) SAINTexpress: improvements and additional features in Significance Analysis of INTeractome software. *J Proteomics* 100: 37-43

Yeh C, Coyaud E, Bashkurov M, van der Lelij P, Cheung SW, Peters JM, Raught B, Pelletier L (2015) The Deubiquitinase USP37 Regulates Chromosome Cohesion and Mitotic Progression. *Curr Biol* 25: 2290-9
Zybailov B, Coleman MK, Florens L, Washburn MP (2005) Correlation of relative abundance ratios derived from peptide ion chromatograms and spectrum counting for quantitative proteomic analysis using stable isotope labeling. *Anal Chem* 77: 6218-24

2nd Editorial Decision 29th Apr 2019

Thank you for submitting your revised manuscript to The EMBO Journal. Referee 2 has now assessed it once more, and I am happy to say finds the original criticisms overall well-addressed. S/he nevertheless retains a number of specific concerns, which I feel shall be important to address by way of a final round of minor revision.

In addition, please kindly also address the following editorial issues at this stage.

After this final round of modification, we should be able to proceed with eventual acceptance and publication of the study. Should you have any additional questions in this regard, please do not hesitate to contact me.

REFEREE REPORT:

Referee #2:

The revised manuscript by Gheiratmand and colleagues addresses many of my original concerns. I am particularly pleased that the authors took on board my suggestion of depleting the centriolar satellite scaffolding component PCM1. What remains are mostly clarifications to the text (see below).

Main points
1. In my original review (point 4) I asked the authors to clarify whether the varying degree of colocalization they observe between PCM1 and various other satellite components is due to those other components localizing to another structure, in particular the centrosome. This should be easily testable by excluding the centrosome from their colocalization analysis. If the authors do want to make a case for satellite populations independent of PCM1, I would ask them to reanalyze their existing data in this manner. Since this does not require new data acquisition I do not see why this should unduly delay their manuscript. At present I am less than fully convinced that non-PCM1-containing satellites exist, particularly since all satellites appear to be sensitive to PCM1 depletion (if there were any satellites not containing PCM1, why should this be?).

2. Another point from my original review that I consider to be only partly addressed is whether there any proteins or protein interactions unique to either satellites or centrosomes (point 12). There clearly is quite a high degree of overlap (p10), which is why I would be interested in the authors' opinion on what defines each cellular structure besides the presence of PCM1 on satellites but not centrosomes. The loss of specific interactions in PCM1-depleted cells (Fig 5) may indicate that there are interactions unique to satellites, unless that loss reflects loss of those proteins also from centrosomes. At least a brief discussion of this question informed by their data would be of great interest to the reader.

Other points
3. Abstract (p2): 'We further show that satellites are lost in PCM1 null cells, reflected by a dramatic change in the interactomes of satellite components.'
The key finding is not that satellites are lost in PCM1-depleted cells, which is to be expected, it is that the interactome is altered. Hence perhaps: 'We further show that loss of satellites in PCM1-

depleted cells results in a dramatic change in the interactomes of satellite components.'

4. Introduction (p4): 'For example, both the depletion and overexpression of CEP290 results in PCM1 redistribution (Kim et al., 2008) and knockdown of SSX2IP or PIBF1 leads to a reduction of PCM1 levels at basal bodies and satellites (Kim et al., 2012, Klinger, Wang et al., 2014).'
Neither Kim et al 2012 nor Klinger et al 2014 demonstrated any reduction in PCM1 levels at satellites. Indeed, I am not aware of any perturbation other than PCM1 depletion that does anything other than disperse satellites.

5. Introduction (p6): 'Proximity-dependent biotin identification (BioID) has emerged as a powerful tool to study centrosome and centriolar satellite proteins (Gupta et al., 2015, Roux, Kim et al., 2012)...BioID has already been successfully applied to a number of studies involving centrosomal and centriolar satellite proteins (Comartin, Gupta et al., 2013, Conkar et al., 2017, Firat-Karalar et al., 2014, Firat-Karalar & Stearns, 2015, Gupta et al., 2015)..'
These two paragraphs are somewhat repetitive. I would make the first about centrosomes and cilia in general and only bring up satellites in the following paragraph.

6. Results (p8): 'We confirmed that inducing the expression of the tagged bait proteins for 24h does not affect satellite morphology, based on PCM1 intensity and the total area that satellites occupy.'
This should be 'distribution' or 'localization', not 'morphology'. Satellites don't have much of the latter.

7. Results (p8/Fig 1C):
I'm still not a fan of the color scheme used to classify prey proteins. What I believe is quite misleading is to color any protein as 'satellite' if it also localizes and functions elsewhere (eg C2CD3, a centriolar protein that regulates centriole elongation/ciliogenesis). As the authors demonstrate many of the proteins depicted here localize to satellites, not just the ones highlighted in yellow. Unless a protein exclusively localizes to satellites, like PCM1, the 'other' localization would be better used for classification.

8. Results (p9): 'Disease genes related to microcephaly and ciliopathy are also found in our network (Fig 1C-D), further confirming the connection between these diseases and satellite/centrosome components.'
The microcephaly/ciliopathy link to centrosomes was already known. What is potentially novel is the link to satellites.

9. Results (p16):
It is somewhat confusing for the authors to describe a heterogeneous population of wild-type and CRISPR-targeted cells as 'PCM1 knockout' cells, as those wild-type cells contribute a significant amount of PCM1 to the extracts used for BioID (see Fig 5A), while clonally selected cells are indeed null (Appendix Fig S5). 'PCM1 depleted' may be a better term to use. This also would avoid odd sentences like 'PCM1 signal was appreciably reduced in PCM1 KO cells' (p16).

10. Results (p18): 'We observed that centrosome depletion in these cells resulted in less pronounced MT organizing centers (MTOC) (Fig EV5A-B), in a manner comparable to the elimination of centrioles via laser ablation (Khodjakov, Cole et al., 2000).'
This should read 'microtubule organization', not 'microtubule organizing centers'. No MTOCs exist in centriole-depleted cells.

11. Discussion (p24/25):
The discussion of a potential link between P body proteins and satellites is somewhat overly long, considering that none of the proteins discussed here were further characterized in the present study.

12. Discussion (p24/25): 'It was recently suggested that PLK4 participates in the regulation of satellite integrity through phosphorylation of PCM1 (Hori, Barnouin et al., 2016), raising the possibility that a satellite, centriole, and P-body axis controls satellite integrity.'
I'm not sure what to make of a 'satellite, centriole, and P-body axis'. Also, how do the authors square the Hori et al result (PLK4 regulates satellite integrity) with the apparent lack of an effect the authors observe of centrinone on the satellite interactome?

13. Discussion (p27): 'Interestingly, even though satellites were disrupted, a large number of proximity interactions persisted, indicating that these protein complexes can assemble in a PCM1-independent manner.'
Or these interactions (also) occur at centrosomes..

General comment
14. Is it truly necessary to have Supplementary Figures and Appendix Supplementary Figures? This is quite confusing for the reader. If this is due to a limitation on the number of Supplementary Figures, I would suggest that the EMBO Journal reconsider their policy.

2nd Revision - authors' response 9th May 2019

Below is our detailed point-by point response (our responses are in bold and the original comments in their entirety are in *italic*). The editor's comments and those of Reviewer#2 have been very helpful and we hope you will now find the revised version of our manuscript suitable for publication in *EMBO J.*

Editorial points

The authors performed all requested editorial changes.

Referee #2:

The revised manuscript by Gheiratmand and colleagues addresses many of my original concerns. I am particularly pleased that the authors took on board my suggestion of depleting the centriolar satellite scaffolding component PCM1. What remains are mostly clarifications to the text (see below).

Main points

1. In my original review (point 4) I asked the authors to clarify whether the varying degree of colocalization they observe between PCM1 and various other satellite components is due to those other components localizing to another structure, in particular the centrosome. This should be easily testable by excluding the centrosome from their colocalization analysis. If the authors do want to make a case for satellite populations independent of PCM1, I would ask them to reanalyze their existing data in this manner. Since this does not require new data acquisition I do not see why this should unduly delay their manuscript. At present I am less than fully convinced that non-PCM1-containing satellites exist, particularly since all satellites appear to be sensitive to PCM1 depletion (if there were any satellites not containing PCM1, why should this be?).

We thank the reviewer for the clarification as we misunderstood his/her original point. The referee is concerned that our intensity correlation profile may have been biased due to the localization of satellite proteins to the centrosomes or its immediate vicinity. This is a good point which we have now addressed quantitatively. As suggested, we re-analyzed the original dataset in its entirety (1125 images) using a modified MATLAB analysis routine that excluded the centrosomal region. This was carried out using gamma-tubulin labeling to define the position of the centrosome, and using that coordinate to generate a mask of approximately 6μm².This is now detailed in the Appendix methods section. Our analysis revealed that using this method, ~10-20% of the total signal was lost with some variation depending on the centriolar satellite protein analyzed (see Appendix Fig S4C). This analysis led to a predictable decrease in intensity correlation overall but nonetheless the general trend previously observed was maintained (e.g. PCM1/CEP131 signals correlate much more than PCM1/CEP290) (see Appendix Fig S4D). Thus, excluding the centrosomal region from our analysis confirms our initial observation that some centriolar satellite proteins colocalize much less with PCM1 than others. We have modified Fig 4C to highlight some examples of this (zoomed in boxes), where we can clearly see, for example, CEP290 satellites that label very poorly with PCM1, which we do not frequently observe with CEP131. We note that this is particularly evident in cells treated with nocodazole where satellites are dispersed (Fig 7C). Furthermore, we would like to emphasize that cells depleted of centrioles using centrinone also display intensity correlation profiles consistent with the idea that some centriolar satellite structures contain

variable amounts of PCM1 in the absence of an MTOC, which is an orthogonal way to mitigate potential bias caused by the localization of satellite proteins to the centrosomes or its immediate vicinity (Fig 7C). We have highlighted these by adding arrowheads to Fig7C.

This reviewer questions the existence of non-PCM1-containing satellites, since all satellites appear to be sensitive to PCM1 depletion and if this was the case, why would any satellites not containing PCM1, be sensitive to its depletion? An alternate possibility could be that PCM1 is necessary for the formation of centriolar satellite structures and once formed it is only over time that these structures lose PCM1. Alternatively, this could also be caused by yet unappreciated dynamic properties of PCM1 that are difficult to capture in end-point assays like those we report in this study. More work will be required to address these salient questions. This is now discussed briefly on page 26 of the revised manuscript.

2. Another point from my original review that I consider to be only partly addressed is whether there any proteins or protein interactions unique to either satellites or centrosomes (point 12). There clearly is quite a high degree of overlap (p10), which is why I would be interested in the authors' opinion on what defines each cellular structure besides the presence of PCM1 on satellites but not centrosomes. The loss of specific interactions in PCM1-depleted cells (Fig 5) may indicate that there are interactions unique to satellites, unless that loss reflects loss of those proteins also from centrosomes. At least a brief discussion of this question informed by their data would be of great interest to the reader.

We thank the reviewer for clarifying this issue further and apologize for not fully addressing it in the first round since we were not 100% certain what s/he was after (former Point#12). We had included some data addressing this point in the revision but obviously we were not sufficiently clear. We included a comparison of the BioID data from our earlier paper (Gupta et al.), consisting of the interactome of 31 centrosome baits versus the centriolar satellite baits analyzed here (Appendix Fig S7, Table EV10 - ciliary transition zone baits were not included in this comparison). This analysis thoroughly compares the overlapping preys between these two datasets and highlights the interactions unique to either satellites or centrosomes which is addressing the reviewer's point. Overall, 34.3% of the 30 bait centrosomal interactome (Gupta) were also detected in the satellite bait interactome. Conversely, 39.9% of the satellite interactome overlaps with the centrosome dataset. Thus, while significant overlap is observed between the two interactomes, the majority of each dataset is specific to the centrosome or satellite. To make this point more clearly, we have also highlighted several examples of "organelle-specific" prey proteins that are detected by 4-5 bait proteins uniquely in either the centriole or satellite (Appendix Fig S7).

Interestingly, the satellite "core" proteins (i.e. those 84 prey polypeptides detected by 11 or more satellite bait proteins) did share significant overlap with the centrosome interactome, suggesting that these highly connected proteins could play important roles in both structures. We have added this statement to the manuscript on Page 28.

Furthermore, we also noted that the 20 centriolar and centriolar appendage baits present in our satellite interactome are not lost upon centriole depletion indicating that their localization to satellites is centrosome-independent. This is now discussed on Page 28 of the revised manuscript. In addition, we found that even though satellites were disrupted, a large number of proximity interactions persisted, indicating that these protein complexes can either assemble in a PCM1-independent manner or that these interactions can also occur at centrosomes. This is mentioned on Page 27 of the revised manuscript.
Together these data indicate that although these two organelles are vastly interconnected, a portion of the proximity interactions observed between their resident proteins are to some degree independent of each other.

The referee mentions that he/she would be interested in our thoughts about what defines each cellular structure besides the presence of PCM1 on satellites but not centrosomes. Our opinion is that by solely relying on unique proximity interaction profiles of either satellites or centrosomes, it would be difficult to define unambiguously compartment specific components. To do this accurately would require using orthogonal methods (e.g. biochemical purification followed by mass spectrometry, as per Gergely et al.) or the use of more conventional affinity purification methods followed by mass spectrometry. This is now mentioned in the discussion on Page 29.

Other points

3. Abstract (p2): 'We further show that satellites are lost in PCM1 null cells, reflected by a dramatic change in the interactomes of satellite components.' The key finding is not that satellites are lost in PCM1-depleted cells, which is to be expected, it is that the interactome is altered. Hence perhaps: 'We further show that loss of satellites in PCM1-depleted cells results in a dramatic change in the interactomes of satellite components.'

Thank you for the comment. The sentence has been changed as suggested.

4. Introduction (p4): 'For example, both the depletion and overexpression of CEP290 results in PCM1 redistribution (Kim et al., 2008) and knockdown of SSX2IP or PIBF1 leads to a reduction of PCM1 levels at basal bodies and satellites (Kim et al., 2012, Klinger, Wang et al., 2014).'
Neither Kim et al 2012 nor Klinger et al 2014 demonstrated any reduction in PCM1 levels at satellites. Indeed, I am not aware of any perturbation other than PCM1 depletion that does anything other than disperse satellites.

This sentence has been rewritten. The new sentence now reads "knockdown of SSX2IP or PIBF1 leads to the dispersion of PCM1 throughout the cytoplasm".

5. Introduction (p6): 'Proximity-dependent biotin identification (BioID) has emerged as a powerful tool to study centrosome and centriolar satellite proteins (Gupta et al., 2015, Roux, Kim et al., 2012)...BioID has already been successfully applied to a number of studies involving centrosomal and centriolar satellite proteins (Comartin, Gupta et al., 2013, Conkar et al., 2017, Firat-Karalar et al., 2014, Firat-Karalar & Stearns, 2015, Gupta et al., 2015)..'
These two paragraphs are somewhat repetitive. I would make the first about centrosomes and cilia in general and only bring up satellites in the following paragraph.

This is a good suggestion and we have modified the manuscript as such on Page 6.

6. Results (p8): 'We confirmed that inducing the expression of the tagged bait proteins for 24h does not affect satellite morphology, based on PCM1 intensity and the total area that satellites occupy.' This should be 'distribution' or 'localization', not 'morphology'. Satellites don't have much of the latter.

We thank the reviewer for pointing this out. 'morphology' has been replaced with 'distribution'.

7. Results (p8/Fig 1C):
I'm still not a fan of the color scheme used to classify prey proteins. What I believe is quite misleading is to color any protein as 'satellite' if it also localizes and functions elsewhere (eg C2CD3, a centriolar protein that regulates centriole elongation/ciliogenesis). As the authors demonstrate many of the proteins depicted here localize to satellites, not just the ones highlighted in yellow. Unless a protein exclusively localizes to satellites, like PCM1, the 'other' localization would be better used for classification.

We apologize for misunderstanding this point the first time around. The referee is correct and we have now changed the labels for the 'centriolar satellites' to 'Both centriolar satellites and centrosomes' and the 'centrosome' to 'centrioles and/or pericentriolar material'. However, we think that having different color schemes for highlighting the proteins that localize to satellites (yellow) in addition to other cellular structures is informative and helps in distinguishing them from proteins that localize to centrosomes only (orange). We have addressed the reviewer's point in the legend by adding this sentence: 'Prey proteins are color coded as indicated. Please note that except for PCM1, which is known to exclusively localize to the centriolar satellites, the rest of the proteins labeled in yellow are known to localize to both satellites and centrioles. Preys marked in orange are known to localize to centrosomes (centrioles and/or pericentriolar material) and not to centriolar satellites, as of the date of this study.'

8. Results (p9): 'Disease genes related to microcephaly and ciliopathy are also found in our network (Fig 1C-D), further confirming the connection between these diseases and satellite/centrosome components.' The microcephaly/ciliopathy link to centrosomes was already known. What is potentially novel is the link to satellites.

This is a good point. 'centrosome' has been deleted from the above sentence to further emphasize the satellites.

9. Results (p16):
It is somewhat confusing for the authors to describe a heterogeneous population of wild-type and CRISPR-targeted cells as 'PCM1 knockout' cells, as those wild-type cells contribute a significant amount of PCM1 to the extracts used for BioID (see Fig 5A), while clonally selected cells are indeed null (Appendix Fig S5). 'PCM1 depleted' may be a better term to use. This also would avoid odd sentences like 'PCM1 signal was appreciably reduced in PCM1 KO cells' (p16).

We agree with the reviewer. 'PCM1 KO' has been replaced with 'PCM1 depleted'. The word 'appreciably reduced' has also been replaced with 'absent'.

10. Results (p18): 'We observed that centrosome depletion in these cells resulted in less pronounced MT organizing centers (MTOC) (Fig EV5A-B), in a manner comparable to the elimination of centrioles via laser ablation (Khodjakov, Cole et al., 2000).'
This should read 'microtubule organization', not 'microtubule organizing centers'. No MTOCs exist in centriole-depleted cells.

This point is well taken and we have edited the manuscript accordingly.

11. Discussion (p24/25):
The discussion of a potential link between P body proteins and satellites is somewhat overly long, considering that none of the proteins discussed here were further characterized in the present study.

This section has been shortened by deleting the following part:
'In the same study using CEP85 as a bait, Youn *et al*. recovered multiple GW/P-body components including GW182 and AGO2, which are also in our dataset, and localize to centrioles (Aizer et al., 2008, Moser et al., 2011, Youn et al., 2018). It was recently suggested that PLK4 participates in the regulation of satellite integrity through phosphorylation of PCM1 (Hori, Barnouin et al., 2016), raising the possibility that a satellite, centriole, and P-body axis controls satellite integrity.'

12. Discussion (p24/25): 'It was recently suggested that PLK4 participates in the regulation of satellite integrity through phosphorylation of PCM1 (Hori, Barnouin et al., 2016), raising the possibility that a satellite, centriole, and P-body axis controls satellite integrity.'
I'm not sure what to make of a 'satellite, centriole, and P-body axis'. Also, how do the authors square the Hori et al result (PLK4 regulates satellite integrity) with the apparent lack of an effect the authors observe of centrinone on the satellite interactome?

As mentioned above, we have removed the discussion about P-bodies and this section has also been removed in the process.

13. Discussion (p27): 'Interestingly, even though satellites were disrupted, a large number of proximity interactions persisted, indicating that these protein complexes can assemble in a PCM1-independent manner.' Or these interactions (also) occur at centrosomes.

We thank the reviewer for this suggestion and now mention 'or these interactions (also) occur at centrosomes' has been added to this section.

General comment

14. Is it truly necessary to have Supplementary Figures and Appendix Supplementary Figures? This is quite confusing for the reader. If this is due to a limitation on the number of Supplementary Figures, I would suggest that the EMBO Journal reconsider their policy.

We have followed the journal guidelines, which impose a limit to the number of the EV figures. We will leave this matter up to the editor.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Laurence Pelletier
Journal Submitted to: EMBOJ
Manuscript Number: EMBOJ-2018-101109R1

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a 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:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was claimed in each figure legend. We randomly selected around the mentioned number of the cell samples in each experiment with three independent repeats to be able to run proper statistical tests and mentioned in each case.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, in most cases t-test was done to identify if there is any signicant difference between the compared samples. Detailed information on the test type and the P-value are mentioned in each figure legends.
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes. The relevant information is described in each Figure legend.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibodies used in this study are all listed in Table EV9 with their producing company and the catalog number or the lab they have been obtained from.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	We've used the following cell lines: hTERT RPE-1 (ATCC) , U-2 OS (ATCC), RPE-1 ΔP53 (Zimmerman et al., 2018), Flp In TREx 293 (Invitrogen). All cell lines are routinely checked in our lab for mycoplasma contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	This part is provided in the manuscript under "Data Availablity": All the mass spectrometry data have been deposited at MassIVE data repository, ID: MSV000083121
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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