EMBO reports

Oncoprotein CIP2A promotes the disassembly of primary cilia and inhibits glycolytic metabolism

Ae Lee Jeong, Hye In Ka, Sora Han, Sunyi Lee, Eun-Woo Lee, Su Jung Soh, Hyun Jeong Joo, Buyanravjkh Sumiyasuren, Ji Young Park, Jong-Seok Lim, Jong Hoon Park, Myung Sok Lee, Young Yang

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(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 23 October 2017

Thank you for your patience while your manuscript was peer-reviewed for EMBO reports. We have now received the enclosed referee reports.

As you will see, the referees acknowledge that the findings are potentially interesting. However, they also raise several concerns that would need to be addressed in order to strengthen the study. Going through the reports, I think that all concerns should be addressed, and especially all missing controls must be provided.

We would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view (EV) figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

Regarding data quantification, please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

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

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

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

REFEREE REPORTS

Referee #1:

Review of Jeong et al "Oncoprotein CIP2A regulates the disassembly of primary cilia and glycolytic metabolism". In this manuscript, the authors investigated whether CIP2A, a centrosomal protein and regulator of the PP2A phosphatase, was important in cilia assembly and in turn for metabolic regulation. CIP2A is an apparent oncogene and might be expected to inactivate cilium when overexpressed. Indeed, they find that CIP2A overexpression induced primary cilia disassembly, which requires Aurora A and Nek2 kinases. The authors found Nek2 by screening all of the Nek kinases, and also find Nek7 and Nek 11 bind CIP2A. Conversely CIP2A depletion increased ciliated cell number and ciliary length in retinal pigment epithelium (RPE) cells. This did not appear to be a direct consequence of cell cycle or autophagy changes, which could have been linked. Using a Seahorse physiometer, the authors show that CIP2A depletion also shifted metabolism toward the glycolytic pathway, and that this was linked to altering the expression of metabolic genes related to glycolysis, including LDHA, which is a HIF1 target. They did not exclude more direct, non-transcriptional effects. Nonetheless, glycolytic activation in CIP2A-depleted cells did not require cilia assembly, although enhanced cilia assembly did somewhat activate glycolytic metabolism. We don't know that much about ciliary disassembly, except for the role of Aurora A. The regulation of AurA by CIP2A is a nice new step in regulation of primary cilia disassembly. Although CIP2A depletion induces metabolic reprogramming independent of primary cilia, it is intriguing to think that there might be a link.

The data are straightforward, and nicely presented with appropriate quantitation and statistics. The effects are more believable as they are clear but not all or nothing (which many labs oversell by making the ciliation data too black and white, usually by choosing short time points). It think the result is of interest to people in the cilia, cell cycle, and centriole fields and suitable for EMBO reports. I don't have any major additional experiments.

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In this manuscript Jeong et al. identify the cancerous inhibitor of protein phosphatase 2A (CIP2A) as a regulator of the disassembly of primary cilia. Cilia are sensory organelles present on many mammalian cells and dysfunction of cilia is causative for many different types of diseases, summarized as ciliopathies. The vast majority of ciliopathies are hereditary syndromes, that overlap phenotypically and genetically and lack any causative therapy. Cilia are nucleated from the basal body, which is a modified mother centriole. This is linking cilia to the cell cycle. A ciliary cell cycle checkpoint has been suggested: cilia are built in G0 and have to be disassembled prior to reentry into mitosis. This fact together with ciliary signaling networks connects cilia to cancer. Consistently, cancer cells typically (with some minor exceptions) show reduced numbers of cilia.

In this study, the authors describe that overexpression of CIP2A induces ciliary disassembly by activating Aurora A. Consistently, the authors observe an increase in ciliated cells and ciliary length when knocking down CIP2A in retinal pigment epithelial cells (RPE1). Independent of primary cilia, they observe a metabolic shift towards glycolysis upon depletion of CIP2A. A similar shift occurs in cells lacking cilia. Taken together, this is an interesting study on an important topic. However, I have some concerns that should be addressed by the authors:

1) Looking at Fig. 1, it will be important to address the question, whether or not the effect of CIP2A depletion on ciliary dynamics is dependent on NEK2 by combining siRNA against CIP2a and NEK2. Later, Fig 3E suggests, that this might not be the case. Moreover, the siRNAs must be validated and the knockdown of CIP2a/Nek2 in the RPEs must be confirmed by WB or at least by qPCR.
2) Another important and easy-to-address question: Where exactly is CIP2A localized at the ciliary base? Adding stainings with additional markers of the ciliary base would be helpful (e.g. pericentrin/gammaTub for the basal body (BB) and Nphp1 or RPGRIP1L as marker for the transition zone (TZ) distal from the BB). This would allow to determine whether CIP2a localizes to BB or TZ or both. It might also be useful to replace "centrosome" in the text and legend by "basal body" or "ciliary base".
3) Given the finding that NEK2 interacts with CIP2A and regulates ciliary dynamics, and that inhibition of NEK2 does not modulated the CIP2A effect (Fig 3E) the exact role of NEK2 is elusive. Is NEK2 required for the localization of CIP2A at the ciliary base or vice versa? Or do NEK2 and CIP2a represent two independent pathways the accidentally collide at the ciliary base? This should be studies or discussed more in detail.
4) The finding that cilia are not associated with the metabolic effects of CIP2a is, although negative, very interesting. However, the ECAR data presented in Fig. 5A (control curve) are different from the curve in Fig 4F. How can this be explained? In addition: Does the knockdown of CIP2A restore ciliogenesis in the CEP290 KO cell lines?
5) More detailed information on the RPE KO cell lines should be provided. How exactly were they generated and validated? Most likely, this was done by Crispr/Cas9 mediated NHEJ. What is the exact INDEL mutation they carry?

Minor points:
1. Introduction, 2nd paragraph: Cilia do not just depend on the cell cycle, but the cell cycle is regulated by cilia. This bi-directional aspect is missing. Introducing a "ciliary checkpoint" would make it easier for the reader the understand the link to cancer. Based on this, the description of Fig. 2 A/B and the conclusion should probably be rephrased.
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5. Fig 1C: In the picture presented GFP alone is also enriched at the centrosome. Is there any explanation?

6. Fig 3A: This CO-IP is lacking important negative controls. To exclude unspecific precipitation IPs with control antibody must be added for every individual NEK subtype. Alternatively, the IP should be performed with anti-GFP, then hunting for CIP2A in the lysates. For this, the experimental design shown in Fig. 3A would fit. In Fig. 3B the negative control for AurA is missing.
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Fig. 3E what is the "control"□ I thought you have a condtion where the inhibitor was not added (0 um).

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2. Knockout or overexpression of an gene may generate novel phenotypes. One critical question is whether this molecule undergoes any changes during physiological conditions.

Overexpression or knockout of CIP2A indeed modulates ciliogenesis. However, I did not find strong evidence that shows physiological relevance. Fig.2B may be the only data to show this. But I think it is not supportive. The size of the bands shown in the input is different from that from IP, so I think these bands are non-specific. For the Co-IP data, because c-tubulin is low in serum starvation and 1.5h, it is really hard to say any changes oeverall (in comparison with the later times). It is better using IgG as control, because c-tubulin may also undergo changes during these conditions.

What I would like to see is that the authors should demonstrate whether CIP2A undergo changes during ciliary assembly or shortening. For example, after serum stimulation, CIP2A may be increased at around the waves of ciliary shortening or decreased during serum starvation.

3. The fucntion of CIP2A is shown by RNAi. The RNAi effect on the proein level of CIP2A should be shown in the formal figures.

4. The data on aurara-A activation is not strong. In Fig. 3D. The loading of Gst-aurora A is more than the rest samples.

5. CIP2A affects metabolism. But ,this work did not reveal how CIP2A regulate metabolism. It seems to me that this part of work is only descriptive. But I agree that it nicely showed that ciliogenesis is not involved.

Referee #1:

Review of Jeong et al "Oncoprotein CIP2A regulates the disassembly of primary cilia and glycolytic metabolism". In this manuscript, the authors investigated whether CIP2A, a centrosomal protein and regulator of the PP2A phosphatase, was important in cilia assembly and in turn for metabolic regulation. CIP2A is an apparent oncogene and might be expected to inactivate cilium when overexpressed. Indeed, they find that CIP2A overexpression induced primary cilia disassembly, which requires Aurora A and Nek2 kinases. The authors found Nek2 by screening all of the Nek kinases, and also find Nek7 and Nek 11 bind CIP2A. Conversely CIP2A depletion increased ciliated cell number and ciliary length in retinal pigment epithelium (RPE) cells. This did not appear to be a direct consequence of cell cycle or autophagy changes, which could have been linked. Using a Seahorse physiometer, the authors show that CIP2A depletion also shifted metabolism toward the glycolytic pathway, and that this was linked to altering the expression of metabolic genes related to glycolysis, including LDHA, which is a HIF1 target. They did not exclude more direct, non-transcriptional effects. Nonetheless, glycolytic activation in CIP2A-depleted cells did not require cilia assembly, although enhanced cilia assembly did somewhat activate glycolytic metabolism. We don't know that much about ciliary disassembly, except for the role of Aurora A. The regulation of AurA by CIP2A is a nice new step in regulation of primary cilia disassembly. Although CIP2A depletion induces metabolic reprogramming independent of primary cilia, it is intriguing to think that there might be a link.

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We thank the referee for the meaningful review and will further elucidate cilia-mediated metabolic reprogramming mechanism and ciliary dynamics regulated by oncoproteins.

Referee #2:

In this manuscript Jeong et al. identify the cancerous inhibitor of protein phosphatase 2A (CIP2A) as a regulator of the disassembly of primary cilia. Cilia are sensory organelles present on many mammalian cells and dysfunction of cilia is causative for many different types of diseases, summarized as ciliopathies. The vast majority of ciliopathies are hereditary syndromes, that overlap phenotypically and genetically and lack any causative therapy. Cilia are nucleated from the basal body, which is a modified mother centriole. This is linking cilia to the cell cycle. A ciliary cell cycle checkpoint has been suggested: cilia are built in G0 and have to be disassembled prior to reentry into mitosis. This fact together with ciliary signaling networks connects cilia to cancer. Consistently, cancer cells typically (with some minor exceptions) show reduced numbers of cilia.

In this study, the authors describe that overexpression of CIP2A induces ciliary disassembly by activating Aurora A. Consistently, the authors observe an increase in ciliated cells and ciliary length when knocking down CIP2A in retinal pigment epithelial cells (RPE1). Independent of primary cilia, they observe a metabolic shift towards glycolysis upon depletion of CIP2A. A similar shift occurs in cells lacking cilia. Taken together, this is an interesting study on an important topic. However, I have some concerns that should be addressed by the authors:

1) Looking at Fig. 1, it will be important to address the question, whether or not the effect of CIP2A depletion on ciliary dynamics is dependent on NEK2 by combining siRNA against CIP2a and NEK2. Later, Fig 3E suggests, that this might not be the case. Moreover, the siRNAs must be validated and the knockdown of CIP2a/Nek2 in the RPEs must be confirmed by WB or at least by qPCR.
Answer: We agree that cotreatment effect of CIP2A and NEK2 siRNAs is informative, thus we have added the result of ciliary dynamics when CIP2A and NEK2 were co-depleted. The result

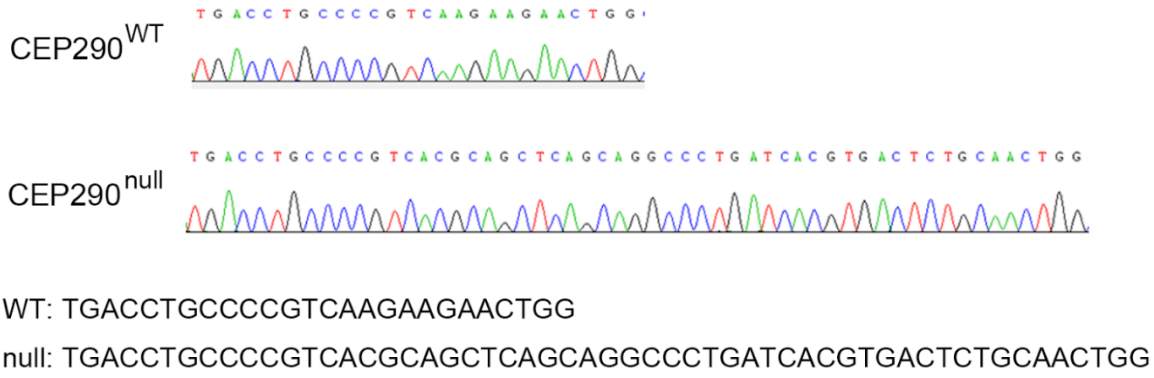
has been updated in revised Fig. 1H and described in the result section on page 6. We were not able to observe additive increase in both CIP2A and NEK2 knockdown.
NEK2 and CIP2A depletion increased the percentage of ciliated cells almost to 80-90 percent, respectively. Thus, we think it is likely hard to further increase the percentage of ciliated cells by both depletion. Therefore, to determine whether cilia disassembly by CIP2A depends on NEK2, CIP2A was overexpressed to reduce the percentage of ciliated cells and then NEK2 inhibitor was treated to examine the percentage of ciliated cells (Fig. 3E and F). Because no change was observed in the percentage of ciliated cells, we think that NEK2 is not associated with CIP2A-mediated cilia disassembly.
We have also validated the depletion effect of CIP2A and NEK2 siRNAs in the RPE1 cells by immunoblot. Data are included in revised Fig. 1F and Fig. 1H.

2) Another important and easy-to-address question: Where exactly is CIP2A localized at the ciliary base? Adding stainings with additional markers of the ciliary base would be helpful (e.g. pericentrin/gammaTub for the basal body (BB) and Nphp1 or RPGRIP1L as marker for the transition zone (TZ) distal from the BB). This would allow to determine whether CIP2a localizes to BB or TZ or both. It might also be useful to replace "centrosome" in the text and legend by "basal body" or "ciliary base".
Answer: To address where CIP2A localizes exactly, we performed staining with markers including γ-tubulin (basal body), CEP164 (distal end of centriole), CEP290 (proximal end of centriole), and TMEM67 (transition zone). As CIP2A co-localized with TMEM67, which is a marker for the transition zone (TZ), we conclude that CIP2A localizes at TZ. We have added the staining results in Fig. 1A and described it on page 5.

3) Given the finding that NEK2 interacts with CIP2A and regulates ciliary dynamics, and that inhibition of NEK2 does not modulated the CIP2A effect (Fig 3E) the exact role of NEK2 is elusive. Is NEK2 required for the localization of CIP2A at the ciliary base or vice versa? Or do NEK2 and CIP2a represent two independent pathways the accidentally collide at the ciliary base? This should be studies or discussed more in detail.
Answer: To address whether CIP2A localization at TZ is NEK2 dependent, we observed CIP2A localization in NEK2 depleted cells or NEK2 inhibitor treated cells. Neither NEK2 depletion nor NEK2 inhibition changed the localization of CIP2A. We have added this additional experiment data on page 8 as Expanded View Fig. 2. Based on this additional result, at least CIP2A and NEK2 are likely to regulate ciliogenesis by two independent pathways.

4) The finding that cilia are not associated with the metabolic effects of CIP2a is, although negative, very interesting. However, the ECAR data presented in Fig. 5A (control curve) are different from the curve in Fig 4F. How can this be explained? In addition: Does the knockdown of CIP2A restore ciliogenesis in the CEP290 KO cell lines?
Answer: We found that extracellular acidification rate (ECAR) is sensitive with serum. When cells are incubated with serum-free media or Hank's Balanced Salt Solution (HBSS), the ECAR data showed a smoothed slope compared with cells with serum-containing media (Referee Fig. 1A). Because RPE1 CEP290 KO cells were serum starved for 24 h in Fig. 4F and cells were serum starved for 48 h in Fig. 5A, it is likely that the ECAR curve shows different slope. In addition, we examined whether ciliogenesis would be restored in CIP2A-depleted RPE1 CEP290 KO cells. However, ciliogenesis restoration was not observed (Referee Fig. 1B).

5) More detailed information on the RPE KO cell lines should be provided. How exactly were they generated and validated? Most likely, this was done by Crispr/Cas9 mediated NHEJ. What is the exact INDEL mutation they carry?
Answer: RPE1 KO cell line was generated by CRISPR/Cas9 mediated NHEJ. INDEL mutation was confirmed by sequencing. Indeed, RPE1 CEP290 KO cells did not express CEP290 protein (Referee Fig. 1C). This work was performed by a collaborator who does not want to use this data before his publication. Thus, we did not add this information in manuscript.



Minor points:
1. Introduction, 2nd paragraph: Cilia do not just depend on the cell cycle, but the cell cycle is regulated by cilia. This bi-directional aspect is missing. Introducing a "ciliary checkpoint" would make it easier for the reader the understand the link to cancer. Based on this, the description of Fig. 2 A/B and the conclusion should probably be rephrased.
Answer: We have included the aspect that referee #2 suggested in the introduction part as below on page 3.
Primary cilia are decreased or lost in a variety of cancer types, including breast, prostate, ovarian, skin, and pancreatic cancer and in renal cell carcinoma and basal cell carcinoma [1-6]. In general, it was considered that the appearance and disappearance of primary cilia passively depend on the cell cycle [7, 8]. However, recent studies have shown that primary cilia do not just depend on cell cycle and actively act as a checkpoint to prevent cell cycle re-entry [9, 10]. Therefore, it is assumed that the absence of the primary cilia may promote tumorigenesis by dysregulating the cell cycle.

Also, as the referee recommended, we have rewritten the description of Fig. 2A and B in the result section as below on page 6.

We next determined whether CIP2A-mediated cilia dynamics could regulate the cell cycle. No significant change in cell cycle distribution was observed in either CIP2A-depleted (Fig. 2A) or CIP2A-overexpressing (Fig. 2B) cells. Because ciliary dynamics are regulated by the cell cycle as well as other factors, it is conceivable that CIP2A-mediated ciliogenesis is not closely related to cell cycle progression.

2. Introduction, 2nd paragraph: For the at hand paper, the landmark study of Pugacheva et al. might be as relevant as the later work on Nek2 and Kif24 (Ref 18). Thus, I would suggest to rephrase this part and highlight the work on AurA/HEF (Ref 19).
Answer: We thank the referee for pointing this issue. We have highlighted the work on Aurora A and HEF first in the revised manuscript.

3. Introduction: The introduction is a little "jumpy". Why not putting the part on the Warburg effect (first part of 3rd paragraph) to the end or to results/discussion?
Answer: As the referee suggested, we have changed the order of the Introduction part as below on page 4.
A decade ago, cancerous inhibitor of protein phosphatase 2A (CIP2A) was revealed as an oncogenic protein overexpressed in most human cancers and is involved in the progression of several human malignancies via the inhibition of protein phosphatase 2A (PP2A) activity, leading to c-MYC stabilization [11]. CIP2A also interacts with NEK2 during G2/M phase and enhances NEK2 kinase activity to facilitate centrosome separation [12]. Because NEK2 regulates cilia disassembly through a concerted mechanism involving Kif24-mediated microtubule depolymerization, we investigated whether CIP2A promotes cilia disassembly. On the other hand, cancer cells efficiently reprogram their metabolism to acquire necessary nutrients and utilize those nutrients for growth and for cellular building blocks, even if oxygen is not limited [13, 14]. This metabolic shift is mediated by the aberrant activation of signaling pathways and oncogenic factors, leading to the altered expression of genes involved in glucose import, catabolism, and anabolism [15]. In this study, since c-MYC is considered a master

modulator of growth and cellular metabolic pathways [16] and CIP2A mediates cancer progression through the metabolic pathway [17], we investigated whether CIP2A affects metabolic reprogramming and whether cilia assembly is able to alter metabolic pathway.

4. Fig 1 B: A negative control for the IP should be provided using a control antibody. Do the signals in the lysates (input) also represent CIP2A? If yes, is there any explanation for the size shift as compared to the precipitates? Please discuss this more detailed in the manuscript.
Answer: We omitted the annotation of control IgG IP sample. We have added the annotation in the revised Fig. 1C. Both input and IP signals in Fig. 1C represent CIP2A. We think that the size difference of CIP2A between IP samples and input may be due to high amount of CIP2A protein in IP samples.
5. Fig 1C: In the picture presented GFP alone is also enriched at the centrosome. Is there any explanation?
Answer: We agree with the referee about the comment. There may be some nonspecific enrichment at the centrosome when GFP is overexpressed. To avoid reader's confusion, we have changed the image in revised Fig. 1D.
6. Fig 3A: This CO-IP is lacking important negative controls. To exclude unspecific precipitation IPs with control antibody must be added for every individual NEK subtype. Alternatively, the IP should be performed with anti-GFP, then hunting for CIP2A in the lysates. For this, the experimental design shown in Fig. 3A would fit. In Fig. 3B the negative control for AurA is missing.
Answer: We absolutely agree with the referee. Thus, we performed the IP experiment again with every individual control. For negative control, pcDNA3.1-FLAG vector was used. Among NEK families, NEK2 interacted most strongly with CIP2A. In addition, CIP2A bound to NEK6, NEK8, NEK9 and NEK11. However, the signal of NEK7 was nonspecific because it was also detected in control. Thus, we have replaced Fig. 3A and described it on page 7.
We have added the negative control for Aurora A in Fig. 3B and replaced it. We appreciate for the critical comments.
7. Result section on Fig 3: The Fig. numbers are mixed up starting with Fig. 3D. 3D must be C, 3E must be D, etc.
Answer: We thank the referee for raising this point. We have corrected the Fig. 3 numbers.
8. MWST are missing and should be added to all immunoblots presented in this study.
Answer: We have now added the molecular weight to all immunoblots in revised figures.
9. I would recommend to shorten the discussion slightly and to remove the speculations on the use of a ketogenic diet "to prevent cancer and improve cancer treatment". It is neither my job nor my responsibility as reviewer. But I would recommend to be more careful at this point. Just imagine that patients (or untalented physicians) read this and draw wrong and probably fatal conclusions.
Answer: As the referee suggested, we have removed the 'ketogenic diet' part in discussion section.
10. 1st line abstract and intro: I would suggest to replace "eukaryotic" by "mammalian". Otherwise the superlative "most" might be debatable.
Answer: We have edited as the referee suggested.

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Answer: We apologize for our insufficient description about the experiment in the figure legends. We have added the detailed information in revised Fig. 1C as below.

RPE1 cells were serum starved (SS) for 24 h, and serum was added for indicated times. For endogenous coimmunoprecipitation analysis, cell lysates were immunoprecipitated (IP) with anti-CIP2A at each time point and IP proteins were analyzed by immunoblot.
Fig. 3E what is the "control"□ I thought you have a condtion where the inhibitor was not added (0 um).
Answer: We apologize for inaccurate description. The meaning of control in Fig. 3E is non-overexpressed cells. To clarify, we have annotated 'non-overexpressed' and 'CIP2A-overexpressed' at the graph. Moreover, figure legend has been updated as below.
The percentage of CIP2A-overexpressed and non-overexpressed cells with primary cilia was evaluated after treatment with the Aurora A inhibitor, MLN 8237 or NEK2 inhibitor, Rac-CCT 250863 (n > 14 cells/condition) under serum-starved condition. The average of three independent experiments is shown, with error bars representing s.d. *p < 0.05 compared with MLN 8237 non-treated cells (one-tailed Student's t-test).

Fig. 4F S.S. was used in describing the conditions. I bet it means serum starvation, it should be explained in the text or figure legend.
Answer: We thank the referee for raising this point. As the referee guessed S.S. means serum starvation and now we have added the description of abbreviations in figure legends.

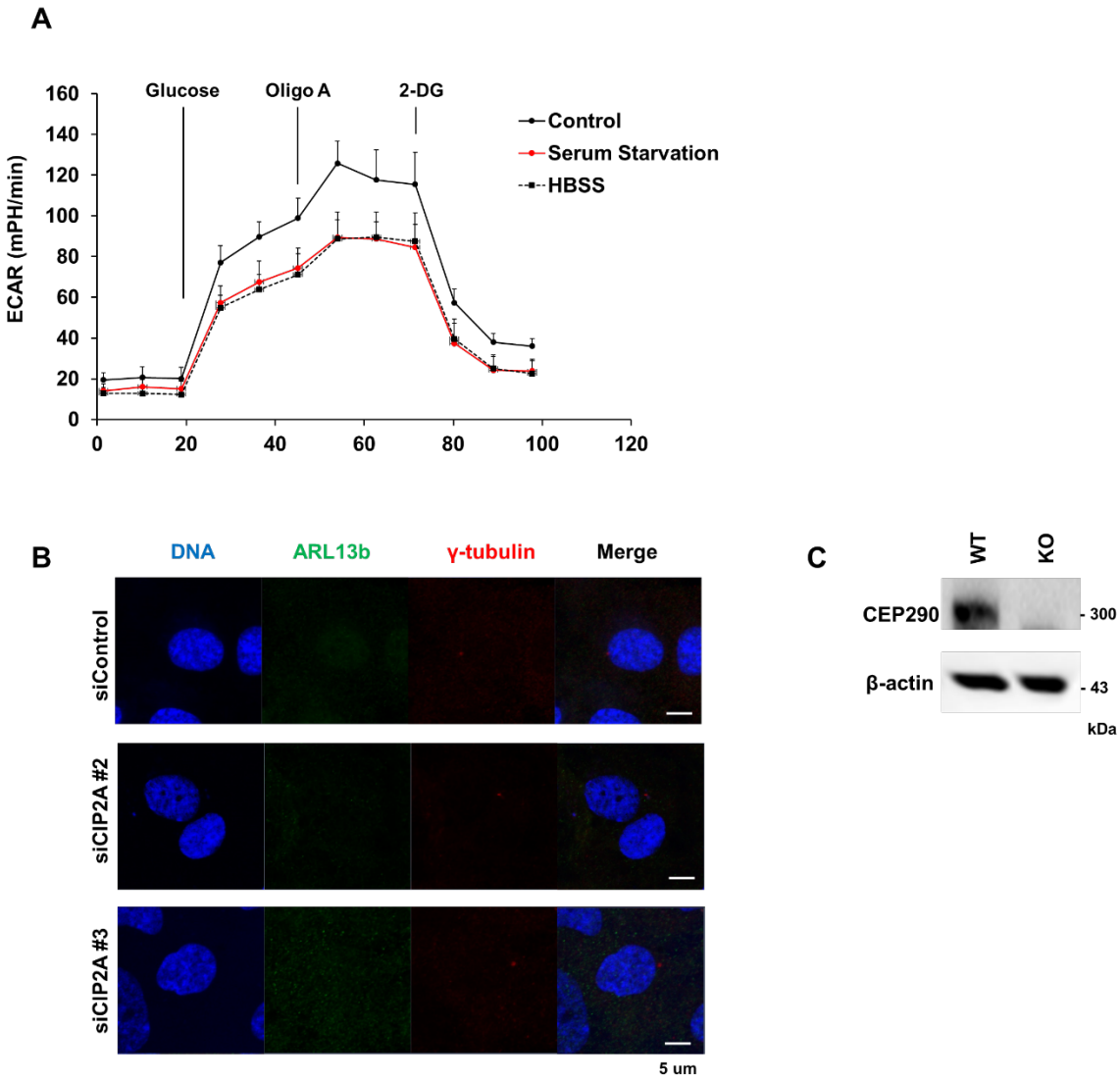
2. Knockout or overexpression of an gene may generate novel phenotypes. One critical question is whether this molecule undergoes any changes during physiological conditions.
Overexpression or knockout of CIP2A indeed modulates ciliogenesis. However, I did not find strong evidence that shows physiological relevance. Fig.2B may be the only data to show this. But I think it is not supportive. The size of the bands shown in the input is different from that from IP, so I think these bands are non-specific. For the Co-IP data, because c-tubulin is low in serum starvation and 1.5h, it is really hard to say any changes oeverall (in comparison with the later times). It is better using IgG as control, because c-tubulin may also undergo changes during these conditions.
Answer: We apologize that we omitted the annotation of control IgG IP sample. We have added the annotation in the revised Fig. 1C. Both input and IP signals in Fig. 1C represent CIP2A. We think that the size difference of CIP2A between IP samples and input may be due to high amount of CIP2A protein in IP samples.
What I would like to see is that the authors should demonstrate whether CIP2A undergo changes during ciliary assembly or shortening. For example, after serum stimulation, CIP2A may be increased at around the waves of ciliary shortening or decreased during serum starvation.
Answer: To find out physiological relevance, we examined changes of CIP2A protein level during serum starvation and re-stimulation. We found that CIP2A decreased after serum starvation and increased after serum re-stimulation by immunoblot and immunostaining analysis (Fig. 1B and Expanded View 1A). Besides, as CIP2A localized at TZ and serum re-stimulation elevated the expression of CIP2A, we tested whether CIP2A accumulate more at TZ after re-stimulation. CIP2A gradually increased after serum re-stimulation and accumulated at TZ to a higher degree at 5 h post re-stimulation by both co-immunoprecipitation and immunostaining (Fig. 1C and Expanded View 1B). These results have been added as a new Fig. 1B and Expanded View Fig. 1A and B.
To further emphasize the physiological relevance, we have added the paragraph in discussion part as below on page 14.
It is known that reduction of cilia length in the hypothalamic neurons of mice increases food intake and decreased energy expenditure, leading to obesity [18]. They found that leptin reduces cilia length in hypothalamic neuronal cells and cilia length is reduced in diet-induced obese (DIO) mice in which leptin level is increased. In this study, we found that serum starved cells show a decrease in CIP2A protein level and serum addition increases CIP2A level. Because CIP2A and cilia length are under the control of nutrient availability in cell level, if we determine whether CIP2A level is upregulated with cilia length decrease in hypothalamic neurons of DIO mice, it is going to be a good evidence to uncover physiological meaning of CIP2A-mediated cilia length control.

3. The function of CIP2A is shown by RNAi. The RNAi effect on the protein level of CIP2A should be shown in the formal figures.
Answer: We have validated the effect of CIP2A siRNAs by immunoblot. Data have been included in revised Fig. 1F and Fig. 1H.

4. The data on aurara-A activation is not strong. In Fig. 3D. The loading of Gst-aurora A is more than the rest samples.
Answer: We thank the referee for bringing this to our attention. We have repeated the experiment and changed it in revised Fig. 3D.

5. CIP2A affects metabolism. But ,this work did not reveal how CIP2A regulate metabolism. It seems to me that this part of work is only descriptive. But I agree that it nicely showed that ciliogenesis is not involved.
Answer: We found that CIP2A increases AP1 transcription factor and AP1 depletion prevented CIP2A-mediated glycolytic gene expression (Fig 5D). Because we did not examine entire transcription factors, we are planning to examine whole transcription factors.

Referee Fig. 1



A. RPE1 cells were seeded onto Seahorse Bioscience V7 cell culture plates (4×10^4/well) followed by changing the media to serum starved-media or HBSS for 48 h. Time course for the measurement of ECAR indicates the basal conditions, followed by the sequential addition of glucose (10 mM), oligomycin (2 μM), and 2-DG (20 mM).
B. CEP290 KO RPE1 cells were transfected with control, CIP2A #2, or CIP2A #3 siRNA. After 24 h, cells were serum starved for 24h and stained with antibodies specific for ARL13B (green), γ-tubulin (red). DNA was stained with DAPI (blue). Shown are the maximum projections from z stacks of representative conditions. Scale bar = 5 μm.

C. WT and CEP290 KO RPE1 cell lysates were analyzed by immunoblot. Following antibodies were used; β-actin (Santa Cruz Biotechnology, sc-47778) and CEP290 (Bethyl Laboratories, A301-659A).

References for revision letter

1. Yuan K, Frolova N, Xie Y, Wang D, Cook L, Kwon YJ, Steg AD, Serra R, Frost AR (2010) Primary cilia are decreased in breast cancer: analysis of a collection of human breast cancer cell lines and tissues. *J Histochem Cytochem* **58**: 857-70
2. Hassounah NB, Nagle R, Saboda K, Roe DJ, Dalkin BL, McDermott KM (2013) Primary cilia are lost in preinvasive and invasive prostate cancer. *PLoS One* **8**: e68521
3. Kim J, Dabiri S, Seeley ES (2011) Primary cilium depletion typifies cutaneous melanoma in situ and malignant melanoma. *PLoS One* **6**: e27410
4. Schraml P, Frew IJ, Thoma CR, Boysen G, Struckmann K, Krek W, Moch H (2009) Sporadic clear cell renal cell carcinoma but not the papillary type is characterized by severely reduced frequency of primary cilia. *Mod Pathol* **22**: 31-6
5. Seeley ES, Carriere C, Goetze T, Longnecker DS, Korc M (2009) Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. *Cancer Res* **69**: 422-30
6. Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH, Jr., Dlugosz AA, Reiter JF (2009) Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med* **15**: 1055-61
7. Nigg EA, Stearns T (2011) The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat Cell Biol* **13**: 1154-60
8. Uetake Y, Loncarek J, Nordberg JJ, English CN, La Terra S, Khodjakov A, Sluder G (2007) Cell cycle progression and de novo centriole assembly after centrosomal removal in untransformed human cells. *J Cell Biol* **176**: 173-82
9. Kim S, Zaghloul NA, Bubenshchikova E, Oh EC, Rankin S, Katsanis N, Obara T, Tsiokas L (2011) Nde1-mediated inhibition of ciliogenesis affects cell cycle re-entry. *Nat Cell Biol* **13**: 351-60
10. Li A, Saito M, Chuang JZ, Tseng YY, Dedesma C, Tomizawa K, Kaitsuka T, Sung CH (2011) Ciliary transition zone activation of phosphorylated Tctex-1 controls ciliary resorption, S-phase entry and fate of neural progenitors. *Nat Cell Biol* **13**: 402-11
11. Junttila MR, Puustinen P, Niemela M, Ahola R, Arnold H, Bottzauw T, Ala-aho R, Nielsen C, Ivaska J, Taya Y*, et al.* (2007) CIP2A inhibits PP2A in human malignancies. *Cell* **130**: 51-62
12. Jeong AL, Lee S, Park JS, Han S, Jang CY, Lim JS, Lee MS, Yang Y (2014) Cancerous inhibitor of protein phosphatase 2A (CIP2A) protein is involved in centrosome separation through the regulation of NIMA (never in mitosis gene A)-related kinase 2 (NEK2) protein activity. *J Biol Chem* **289**: 28-40
13. Warburg O (1956) On the origin of cancer cells. *Science* **123**: 309-14
14. Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. *Nat Rev Cancer* **11**: 85-95
15. Dang CV (2012) Links between metabolism and cancer. *Genes Dev* **26**: 877-90
16. Dang CV (2013) MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* **3**
17. Peng B, Lei N, Chai Y, Chan EK, Zhang JY (2015) CIP2A regulates cancer metabolism and CREB phosphorylation in non-small cell lung cancer. *Mol Biosyst* **11**: 105-14
18. Han YM, Kang GM, Byun K, Ko HW, Kim J, Shin MS, Kim HK, Gil SY, Yu JH, Lee B*, et al.* (2014) Leptin-promoted cilia assembly is critical for normal energy balance. *J Clin Invest* **124**: 2193-7

2nd Editorial Decision 30 January 2018

Thank you for the submission of your revised manuscript. We have now received the enclosed comments from the referees and I am happy to tell you that both support the publication of your revised study now. We can thus in principle accept it.

Only a few minor changes are still needed:

- the manuscript has 5 main figures but is layed out as a full-length article. Please either add one more main figure to the main text or combine the results and discussion sections to convert it into a scientific report. The character count of a short report should not exceed 27.000 characters including spaces but excluding references and materials and methods.

- figures 1-5 often do not specify "n" and the error bars. All figure panels that show graphs with bars need to specify n and error bars. n is the number of independently performed experiments, but the number of cells used can also be given. If the data are based on a single, representative experiment, this also needs to be mentioned. Please add the missing information. I attach a word file of the manuscript here with comments on the figure legends to make it easier, please use this file to work with and send the corrected file back to us.

- in figure 4H, J it is not clear what the little images on the right are, please explain and modify the panels so that it becomes clear what is depicted.
- the panels of figure 4 are not called out alphabetically in the ms text, please correct
- figure 1A - the merge panels in rows 1 and 2 don't seem to be correct
- figure 1D - the merge panel in row 1 doesn't seem to be correct
- figure 3B - The Aurora blot in the source data is missing a band.
- figure EV1B - the middle merge panel is not correct.

I would like to suggest some minor changes to the title and abstract:

Oncoprotein CIP2A promotes the disassembly of primary cilia and inhibits glycolytic metabolism

In most mammalian cells, the primary cilium is a microtubule-enriched protrusion of the plasma membrane and acts as a key coordinator of signaling pathways during development and tissue homeostasis. The primary cilium is generated from the basal body, and cancerous inhibitor of protein phosphatase 2A (CIP2A), the overexpression of which stabilizes c-MYC to support the malignant growth of tumor cells, localizes at the centrosome. Here we show that CIP2A overexpression induces primary cilia disassembly through the activation of Aurora A kinase, and CIP2A depletion increases ciliated cells and cilia length in retinal pigment epithelium (RPE1) cells. CIP2A depletion also shifts metabolism toward the glycolytic pathway by altering the expression of metabolic genes related to glycolysis. However, glycolytic activation in CIP2A-depleted cells does not dependent on cilia assembly, even though enhanced cilia assembly alone activates glycolytic metabolism. Collectively, these data suggest that CIP2A promotes primary cilia disassembly and that CIP2A depletion induces metabolic reprogramming independent of primary cilia.

Please let me know whether you agree with these changes.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the final manuscript.

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I look forward to seeing a final version of your manuscript as soon as possible.
Please let me know if you have any questions or comments.

REFEREE REPORTS

Referee #2:

All critical points I previously raised have been fully addressed by the authors in the revised manuscript. I do not have any additional concern.

Referee #3:

The authors have made extensive revisions and my questions are adequately answered. I recommend publication.

2nd Revision - authors' response 5 February 2018

We have corrected each of the comments by the editor. The major changes are as follows.

1. We have added one main figure to the main text to fit as a full-length article.
2. The missing information about “n” and error bars in figures 1-5 have been specified.
3. All of the merge panels and source data were replaced.
4. We rearranged the panels of figure 4 with the manuscript text.
5. The title and abstract have been changed as the editor suggested.

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: Young Yang
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2017-45144V2

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?	N/A
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No sample was excluded for the analysis.
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.	Yes, fields of immunofluorescence images were randomly selected for each treatment. The statement is included in the Methodes section, Immunofluorescence microscopy.
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Researchers were blinded to treatments while performing cellular analyses.
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	Yes. The appropriate statistical tests were used and stated for each figure.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Because the sample size was small, the type of distribution could not be determined.
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 statistical tests used were stated in figure legends.

C- Reagents

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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).	It is described in Materials and Methods section, Antibodies and chemicals.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	It is described in Materials and Methods section, Cell culture.

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

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
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.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

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	In Material and Methods section, Data Availability statement was included.
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).	N/A
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).	N/A
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.	N/A

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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.	We do not expect this study to fall within dual use research restrictions.