EMBO reports

Casein kinase 1 and 2 phosphorylate Argonaute proteins to regulate miRNA-mediated gene silencing

Vivek Shah, Julia Neumeier, Miguel Huberdeau, Daniela Zeitler, Astrid Bruckmann, Gunter Meister, and Martin Simard
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Corresponding author(s): Martin Simard (martin.simard@crchudequebec.ulaval.ca)

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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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 3rd May 2023

Dear Prof. Simard

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are interesting and both referees are supportive of publication, given that a few issues and concerns are addressed, as outlined in their reports.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) 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.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (August 3rd). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

I am also happy to discuss the revision further via e-mail or a video call, if you wish.

*****IMPORTANT NOTE:
We perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.

2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.*****

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
Please download our Figure Preparation Guidelines (figure preparation pdf) from our Author Guidelines pages https://www.embopress.org/page/journal/14693178/authorguide for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines
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6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their

respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>).

Specifically, we recommend to provide public access to the mass spectrometry data.

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method) that follows the model below (see also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

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Additional information on source data and instruction on how to label the files are available <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

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10) Figure legends and data quantification:
The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
- the nature of the bars and error bars (s.d., s.e.m.)

- If the data are obtained from n {less than or equal to} 5, show the individual data points in addition to the SD or SEM.
- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat

- Please also include scale bars in all microscopy images.

11) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should

directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

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

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

Yours sincerely,

Martina Rembold, PhD
Senior Editor
EMBO reports

****

Referee #1:

The manuscript by Shah et al., identifies kinases that phosphorylate conserved residues on miRNA Argonautes (AGOs). Phosphorylation of AGO has previously been shown to be important for miRNA target regulation in human cell culture and C. elegans worms. Here the authors report that Casein Kinase I alpha (CK1A1) and Casein Kinase 2 (CK2) are responsible for the phosphorylation of specific sites on human AGO2 and worm ALG-1. They use a battery of in vitro and in vivo assays to delineate the phosphorylation sites and corresponding enzyme, as well as present evidence for priming activity needed for sequential phosphorylation at some positions. Additionally, they demonstrate the importance of these sites and kinases for miRNA target regulation in developing C. elegans. Overall, the studies are rigorous and support the conclusions. This work contributes new players and insights to the model describing how post-translational modifications regulate the ability of miRISC to bind, regulate, and release from specific mRNA targets.

Minor points:

1. The title could be improved. For example "a conserved cluster of Argonaute" does not make sense and it is the function of the AGO protein, not the miRNA (whose function is to act as a guide by base pairing).

2. Last sentence in Abstract - add "that" between suggest and phosphorylation

3. Can the authors speculate how kin-19 (RNAi) leads to increased let-7 family miRNA levels?

Referee #2:

Shah et al. describe in great detail how Argonaute phosphorylation is brought about by the kinases CK1A1 (KIN-19) and CK2 (KIN-3/KIN-10). This is important work, because the issue of regulation of Argonaute proteins is still generally poorly understood, and the finer details of how phosphorylation of a known phosphor-cluster proceeds represents an important step forward. Additionally, the authors implicate the modification of this cluster in the mRNA targeting step, which is also a new and important insight.
I overall strongly support publication of this work in EMBO Reports. However, a few, relatively minor, aspects will need to be addressed.

1-the manuscript is in general rather lengthy for the rather straightforward message of the paper. Especially the introduction would benefit from a bit more focus on the relevant issues that are studied. It is not a review we are talking about. The same holds true, however, for the results parts. The manuscript would gain clarity if the authors got to the main issue faster.

2-the authors describe effects on lin-41 on two different occasions. Once with a protein read-out, once with an mRNA readout. It would be better to address these two issues at the same time. When I was reading the results on mRNA effects on page 13 I felt like I had already gotten that result, but at the protein level, on page 11. Merging that could also help shorten the paper.

3-typo page 12 line 6: kin-19 should be kin-10 I guess

4-does the phospho-specific antibody recognize the phosphor-mimic mutation (S992E) in ALG-1? Also, to further demonstrate specificity of the Ab, it would be nice to show that a S998/995/998A triple mutant is still recognized. S992-PO4 is needed, but given that this modification is at the top of a cascade, I think it cannot be excluded yet that the Ab also recognizes phosphorylation of some of the other residues. If so, this should show as a loss of signal by this Ab, if I'm correct. Alternatively, specific peptides should be used to nail down the specificity further.

5-To address better how CK1/2 affect miRNAs a miRNAseq experiment would be great. In the resent manuscript, RT-qPCR is used, which in principle is ok. miRNAseq, however, would be much more inclusive and give a general picture. It would give the paper just that little extra. Not an essential point, however.

6-on page 14 second paragraph, the authors describe that transfected AGO2 is phorphorylated. Yet they also use the same protein from the same source as a substrate in the experiments described just above. Some words on how this makes sense would be good. I guess that it rests on the assumption that only a small fraction is phosphorylated?

7-Figure 7E: is it possible to provide some feeling of data variance here? There is no error-bar or something like that. There should be replicates though, so some statistics should be possible. Without that, it is hard to know how strong the effects really are.

8-Sometimes the phrasing is a bit confusing. Notably when the authors talk about rescuing kin-19 kd effects by mutating alg-1. The authors tend to write that the kin-19 kd rescues the serine mutations, while the text overall talks about these experiments vice versa: the mutations are tested whether they rescue kin-x RNAi. This is a minor point, but it halted me a few times and made me re-read several sections.

Point-by point Responses

We thank all referees for their positive comments and insightful inputs on our manuscript. *Here are the detailed answers to their specific questions:*

Referee #1:

The manuscript by Shah et al., identifies kinases that phosphorylate conserved residues on miRNA Argonautes (AGOs). Phosphorylation of AGO has previously been shown to be important for miRNA target regulation in human cell culture and C. elegans worms. Here the authors report that Casein Kinase I alpha (CK1A1) and Casein Kinase 2 (CK2) are responsible for the phosphorylation of specific sites on human AGO2 and worm ALG-1. They use a battery of in vitro and in vivo assays to delineate the phosphorylation sites and corresponding enzyme, as well as present evidence for priming activity needed for sequential phosphorylation at some positions. Additionally, they demonstrate the importance of these sites and kinases for miRNA target regulation in developing C. elegans. Overall, the studies are rigorous and support the conclusions. This work contributes new players and insights to the model describing how post-translational modifications regulate the ability of miRISC to bind, regulate, and release from specific mRNA targets.

We thank the referee for acknowledging this work's importance and for their positive comments.

Minor points:

1. The title could be improved. For example "a conserved cluster of Argonaute" does not make sense and it is the function of the AGO protein, not the miRNA (whose function is to act as a guide by base pairing).

We agree with the referee's suggestion and have modified the title to "Casein kinase 1 and 2 phosphorylate Argonaute proteins to regulate miRNA-mediated gene silencing".

2. Last sentence in Abstract – add “that” between suggest and phosphorylation

We added “that” between “suggest” and “phosphorylation” in the last sentence of the abstract.

3. Can the authors speculate how kin-19 (RNAi) leads to increased let-7 family miRNA levels?

*We do not know for sure why only CK1A1 depletion (*kin-19 *RNAi) and not CK2 depletion (*kin-3 *and* kin-10 *RNAi) leads to a slight increase in the let-7 family of miRNA levels (which is also observed by the new miRNA sequencing data included in the revised manuscript (new Figure EV4A)). As a certain number of animals always display let-7-related phenotypes (alae defects, seam cells number) upon* kin-19 *knockdown in different genetic backgrounds affecting ALG-1 S992 and S995 phosphorylation (Figure 2), we can postulate that KIN-19 has likely other substrate(s) that are important for the stability and turnover of let-7 family miRNA. Interestingly, we can identify in silico putative phosphorylation sites of CK1A1 in DCS-1 and XRN-1, two controllers of let-7 family miRNA levels in* C. elegans *(Chatterjee et al, 2011; Bossé et al, 2013). We have now included this speculation in the revised manuscript as follows:*

"Intriguingly, we found that the kin-19 RNAi knockdown in wild-type, alg-1(S992E) *and alg-1*(S995E) *animals causes a significant increase in the levels of members of the let-7 family of miRNAs in all strains (Fig 4A-D), which could indicate that KIN-19 has likely other substrate(s) that are important for the stability and turnover of this miRNA family such as DCS-1 and XRN-1, two important controllers of miRNA levels (Bossé* et al*, 2013; Chatterjee* et al*, 2011), which contain consensus CK1A1 phosphorylation sites based on NetPhos 3.1 database (DCS-1: serine at position 63; XRN-1: serine at positions 359, 407, 658 and 1291 and threonine at position 1200)."*

Referee #2:

Shah et al. describe in great detail how Argonaute phosphorylation is brought about by the kinases CK1A1 (KIN-19) and CK2 (KIN-3/KIN-10). This is important work, because the issue of regulation of Argonaute proteins is still generally poorly understood, and the finer details of how phosphorylation of a known phosphor-cluster proceeds represents an important step forward. Additionally, the authors implicate the modification of this cluster in the mRNA targeting step, which is also a new and important insight. I overall strongly support publication of this work in in EMBO Reports. However, a few, relatively minor, aspects will need to be addressed.

We thank the referee for their positive appraisal of our study and for highlighting the relevance of the study.

1-the manuscript is in general rather lengthy for the rather straightforward message of the paper. Especially the introduction would benefit from a bit more focus on the relevant issues that are studied. It is not a review we are talking about. The same holds true, however, for the results parts. The manuscript would gain clarity if the authors got to the main issue faster.

We thank the referee for their proposition. We have shortened the introduction section to focus only on relevant issues for the manuscript and edited the results section as well.

2-the authors describe effects on lin-41 on two different occasions. Once with a protein read-out, once with an mRNA readout. It would be better to address these two issues at the same time. When I was reading the results on mRNA effects on page 13 I felt like I had already gotten that result, but at the protein level, on page 11. Merging that could also help shorten the paper.

We thank the referee for their comment. We agree that the effects on lin-41 *mRNA and protein levels would make more sense if merged in the same section. We have revised the manuscript accordingly and moved Figure 2E and 2F to new panels Figure 5D and 5H, respectively.*

3-typo page 12 line 6: kin-19 should be kin-10 I guess

Thank you for pointing that out. The referee is right; it should be kin-10*. We fixed the typo.*

4-does the phospho-specific antibody recognize the phosphor-mimic mutation (S992E) in ALG-1? Also, to further demonstrate specificity of the Ab, it would be nice to show that aS998/995/998A triple mutant is still recognized. S992-PO4 is needed, but given that this modification is at the top of a cascade, I think it cannot be excluded yet that the Ab also recognizes phosphorylation of some of the other residues. If so, this should show as a loss of signal by this Ab, if I'm correct. Alternatively, specific peptides should be used to nail down the specificity further.

This is an excellent idea from the referee. We have conducted the required experiment and included the results in the revised Figure 3C and the expanded view Figure EV3. The phospho-specific antibody does not recognize the phospho-mimicking S992E mutation on ALG-1.

We used CRISPR/Cas9 to generate the triple mutant of alg-1 (S988A;S995A;S998A) as requested by the referee. This triple mutant only retains the serine at the 992 position and has the other 3 serines mutated to alanines. The phospho-specific antibody does indeed recognize this triple mutant, however, the signal is remarkably reduced when compared to ALG-1 WT (new Figure 3C). This new data aligns with the referee's prediction that the antibody would also recognize the other residues within the cluster due to the S992-PO4 being at the top of the cascade. This experiment also supports the idea that the S992 site initiates hierarchical phosphorylation of the other residues within the cluster. When the other serines are mutated to alanines (triple mutant), there is a significant loss of signal with this antibody. This new data has now been included and discussed in the revised manuscript.

5-To address better how CK1/2 affect miRNAs a miRNAseq experiment would be great. In the recent manuscript, RT-qPCR is used, which in principle is ok. miRNAseq, however, would be much more inclusive and give a general picture. It would give the paper just that little extra. Not an essential point, however.

We thank the referee for this suggestion that a miRNA-seq experiment would give a general idea of how CK1A1 and CK2 depletion affects miRNAs globally. We conducted a miRNA-seq experiment on wild-type animals to assess the global miRNA abundance upon CK1A1 and CK2 RNAi knockdown. We have now included these results in the new expanded view Figure EV4 (A, B, and C). We found that consistent with our RT-qPCR experiments, some members of the let-7 family of miRNAs (let-7 and miR-48) are slightly increased upon kin-19 *RNAi. We also noted that the global miRNA abundance is unaffected upon CK1A1 and CK2 depletion. This is now included in the revised manuscript.*

6-on page 14 second paragraph, the authors describe that transfected AGO2 is phosphorylated. Yet they also use the same protein from the same source as a substrate in the experiments described just above. Some words on how this makes sense would be good. I guess that it rests on the assumption that only a small fraction is phosphorylated?

We thank the referee for pointing this out. Indeed, the reviewer is correct, and we assume that there is enough unphosphorylated AGO2 available for the reaction. We reported in our initial study that about 10-15% of cellular AGO2 is phosphorylated. For clarification, we added the following sentence to the text:

"Since our previous quantifications revealed that about 10-15% of cellular AGO2 is phosphorylated (Quévillon Huberdeau et al, 2017) a substantial amount of unphosphorylated AGO2 should remain as potential substrate for CK1A1."

7-Figure 7E: is it possible to provide some feeling of data variance here? There is no error-bar or something like that. There should be replicates though, so some statistics should be possible. Without that, it is hard to know how strong the effects really are.

We apologize that we did not include statistics in our previous figure. We now included two more biological replicates and added error bars for the percental phosphorylation in the diagram. This should provide some feeling for the variance of cluster phosphorylation between different biological replicates. The addition of more replicates does not change the observed effects and our conclusions.

8-Sometimes the phrasing is a bit confusing. Notably when the authors talk about rescuing kin-19 kd effects by mutating alg-1. The authors tend to write that the kin-19 kd rescues the serine mutations, while the text overall talks about these experiments vice versa: the mutations are tested whether they rescue kin-x RNAi. This is a minor point, but it halted me a few times and made me re-read several sections.

We are sorry for the confusion. We have adjusted the phrasing throughout the revised manuscript.

REFERENCES:

Bossé GD, Rüegger S, Ow MC, Vasquez-Rifo A, Rondeau EL, Ambros VR, Grosshans H & Simard MJ (2013) The decapping scavenger enzyme DCS-1 controls microRNA levels in Caenorhabditis elegans. *Mol Cell* 50: 281–287

Chatterjee S, Fasler M, Büssing I & Grosshans H (2011) Target-mediated protection of endogenous microRNAs in C. elegans. *Dev Cell* 20: 388–396

Quévillon Huberdeau M, Zeitler DM, Hauptmann J, Bruckmann A, Fressigné L, Danner J, Piquet S, Strieder N, Engelmann JC, Jannot G, *et al* (2017) Phosphorylation of Argonaute proteins affects mRNA binding and is essential for microRNA-guided gene silencing in vivo. *EMBO J* 36: 2088–2106

1st Revision - Editorial Decision **17th Aug 2023**

Dear Prof. Simard

Thank you for the submission of your revised manuscript to EMBO Reports. We have now received the report from referee #2 who was asked to assess it.

As you will see, the referee supports publication, given that the discrepant results regarding let-7 regulation are resolved, as suggested by the referee. Please also provide a point-by-point response to this remaining concern.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

- Please remove the Author Contributions from the manuscript file and make sure that the author contributions in our online submission system are correct and up-to-date. The information you specified in the system will be automatically retrieved and typeset into the article. You can enter additional information in the free text box provided, if you wish.

- Please move the Data availability section to the end of 'Material and Methods".

- Please do not forget to remove the referee tokens from the Data availability section.

- It seems that you have reused images shown in Figure 2 A,B,C,D in Figure EV2 Part 1 and Part 2. Please clearly state this in the legends of Figure 2 and EV2. If these are representative examples, I suggest replacing these with other images to show the variance of phenotypes. Otherwise, please state the re-use.

- Tables EV1-3 need legends added to the corresponding files.

- Table EV4 should be made Dataset EV1 and needs the title added to the tab with the description. Please also update the callouts in the text. The file is uploaded as file type 'Dataset".

- Figure EV2 is presented as spanning over two different pages. This will result in the partial display of the figure in the final version of the article. Please provide a one-page Figure EV2 or split the image in two Figures, Figure EV2 and EV3 with consequent renumbering of the following figures. But note that we can only typeset up to 5 EV figures.

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I look forward to seeing a final version of your manuscript as soon as possible.

Kind regards,

Martina Rembold, PhD
Senior Editor
EMBO reports

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Referee #2:

The authors have addressed my concerns adequately. However, I do have one issue that relates to the new small RNA sequencing data in relation to the qPCR data that was shown initially (and which showed a minor upregulation of let-7 and some let-7 family members).

The authors mention in the rebuttal that a small upregulation of let-7 is detected by the sequencing, consistent with the qPCR. However, looking at the data, I have a hard time believing that let-7 is indeed up in the sequencing data (no statistics provided, but the data look very sharp). In the manuscript the authors do not mention this and they only refer to the sequencing data in light of 'no overall miRNA changes'. This is correct.

However, the authors do keep referring to the qPCR result, which claims elevated let-7 levels, and do not relate that to the sequencing data. I would argue that the qPCR method detects a minor decrease in expression of sn2841, which was used for normalization, rather than a small increase in let-7; especially since the authors do not have a miRNA qPCR that does not show upregulation.

One may argue which method is better suited for miRNA expression, qPCR or sequencing, but one cannot ignore contradicting results from two methods. I would go with the sequencing, because the normalization is based on a much broader set of transcripts. This leads to the conclusion that there is no effect on miRNA levels. I do not believe that this conclusion contradicts any of the findings and I do not see the point of keeping in the qPCR data. But if both are kept, the discrepancy needs to be addressed.

Point-by-point Responses

We thank the reviewer for supporting our revised manuscript for publication at EMBO

Referee #2:

The authors have addressed my concerns adequately. However, I do have one issue that relates to the new small RNA sequencing data in relation to the qPCR data that was shown initially (and which showed a minor upregulation of let-7 and some let-7 family members).

The authors mention in the rebuttal that a small upregulation of let-7 is detected by the sequencing, consistent with the qPCR. However, looking at the data, I have a hard time believing that let-7 is indeed up in the sequencing data (no statistics provided, but the data look very sharp). In the manuscript the authors do not mention this and they only refer to the sequencing data in light of 'no overall miRNA changes'. This is correct.

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We are glad that we were able to address all referee's concerns adequately. It is true that comparing RT-qPCR and small RNA sequencing is always difficult (especially when the effects detected are minor) as both methods use way different numbers of normalizers. As suggested by the reviewer, we have decided to keep only the small RNA sequencing data and remove the RT-qPCRs in the manuscript, as they provide a broader survey of the miRNAs levels upon the knockdown of the CK1A and CK2 orthologs, which is more informative to understand the contribution of those kinases in the microRNA pathway. As a result, we have now moved the sequencing data into the main figure (new Figure 4) and remove the RT-qPCR data from the manuscript. We also modified the Result Section accordingly.

Prof. Martin Simard
Oncology-CHU de Québec-Université Laval Research Centre
9 McMahon
Quebec City, Quebec G1R 3S3
Canada

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

Kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

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Reporting Checklist for Life Science Articles (updated January
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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;
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Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods, Figures

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