THE EMBO JOURNAL

SUMOylated PRC1 controls histone H3.3 deposition and genome integrity of embryonic heterochromatin

Zichuan Liu, Mathieu Tardat, Mark E. Gill, Helene Royo, Raphael Thierry, Evgeniy A. Ozonov, Antoine H.F.M Peters

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Editor: Stefanie Boehm

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 9 November 2019

Thank you for submitting your manuscript on SUMO-PRC1 mediated control of genome integrity for consideration by The EMBO Journal. We have now received three referee reports on your study, which are included below for your information.

As you will see, the reviewers are overall positive and acknowledge the interest and quality of the study. Nonetheless they also raise some concerns that would need to be addressed experimentally or discussed in a revised manuscript. Should you be able to adequately do so, we would be happy to consider this study further for publication. Therefore I would like to invite you to prepare and submit a revised manuscript. Please note that it is our policy to allow only a single round of major revision and that it is therefore important to clarify all key concerns raised at this stage.

REFEREE REPORTS

Referee #1:

The manuscript "SUMO-PRC1 controls genome integrity by DAXX-mediated H3.3 incorporation in embryonic heterochromatin" by Liu et al. describes complex mechanism of DAXX-mediated control of H3.3 deposition into the pericentromeric heterochromatin (PCH) in paternal pronuclei of mouse zygotes. The authors have demonstrated that the process is tightly controlled by sumolyation of CBX2 (the component of Polycomb Repressive Complex, PRC1), catalysed by SUMO2. The sumolyated CBX2, in turn, directly interacts with DAXX and facilitates the deposition of H3.3 into the target genomic loci. The presence of H3.3-containing nucleosomes directly provides chromosomal stability during the first embryonic cell division, thus imposing biological impact on

early embryonic development. The provided experimental data demonstrate the dual role of DAXX on genome activity: it's deficiency results in reduced transcription of major satellite repeats, but up-regulates the expression of some genes, related to development and differentiation.

The manuscript is clearly structured and well written, allowing the reader to understand rather complex interactions between the multiple components, regulating and mediating DAXX activity. The experimental data and the logical rationale behind the experiments are solid, as well as the statistical evaluation of the data.

There are a few minor points which still should be addressed:

Figure 1A. The zygote in the middle panel looks more like PN1, rather than PN2/3 - which means that the DNA replication has not even started yet. Also the presence of H3.2-GFP signal inside the pronuclei does not automatically imply the incorporation of the histone into the chromatin.
Figure 6B is missing.
In figures descriptions - what is the difference between "WT" and "Daxx I suppose that both refer to WT embryos.
It is curious, why the levels of Daxx mRNA are also strongly reduced in Daxx m-z- embryos? There should be only exon 2 missing, but the rest of mRNA could still be mappable?

Referee #2:

This interesting paper addresses how the chromatin landscape is established in the pericentric heterochromatin (PCH) of paternal chromosomes in mouse pre-implantion embryos. Whereas the female genome is maintained as chromatin from oocyte to zygote, the paternal genome in sperm is largely devoid of histones and instead is packaged using protamines and on fertilisation the paternal chromatin needs to be re-established. Whereas the maternal PCH is marked by histone H3K9me and HP1, the paternal PCH contains the replacement histone variant H3.3. This research group, in previous publications, have determined many of the steps in this process and this report concentrates on the role of the histone H3.3 chaperone Daxx. Here they show the importance of paternal genome-specific sumoylation with SUMO2/3 of CBX2, a component of the polycomb complex PRC1. This drives recruitment of Daxx via its sumo interacting domains which in turn leads to H3.3 deposition. This process of establishing chromatin is needed to maintain genomic stability and in its absence there is decompaction of PCH, chromosome breakage and mis-segregation. A surprising result given that Polycomb represses transcription of PCH was the finding that knocking out Daxx itself led to substantial repression of transcription. This leads them to conclude that though Daxx works in the same pathway as Polycomb their opposing roles must be involved in balancing expression.

This is a very comprehensive study, the experiments are well designed and executed and data is clear and persuasive. One criticism is that the relevance of this work in the field is skated over. It is also very densely written and this contributes to a rather hard read. One area not explored is whether ATRX, a chromatin remodeller and a partner of Daxx, plays a role in establishing the paternal PCH chromatin. However this is probably beyond the scope of this report and could be a focus of future research.

Minor points

In Fig 6B data is missing.

There are no legends associated with the movies.

Figure 6H the reduction in H3.3-EGFP fluorescent signal is said to be "major" but I think this is rather subtle and it is difficult to define the nucleolar body especially in the Daxxm-z+. It might help if a Dapi stain was included.

Where is the ontology analysis mentioned on p13 line3?

The data in Fig 7J is presented in a rather odd way - why not present in the same way as Figs 3F-H?

Referee #3:

In this manuscript, Liu et al. investigated the maternal function of Daxx, an H3.3 chaperone, on the formation of pericentromeric heterochromatin (PCH).
In addition to their previous paper (Tardat et al., 2015 Mol Cell) which built up a PRC1-Cbx2 pathway in paternal PCH and Suv39h2-HP1 in the maternal PCH, the authors provided new pieces of evidence of their interaction with Daxx/ATRX-H3.3 complex via SUMOylating.
The differences in recruitment of Daxx and ATRX between maternal PCH and Paternal PCH also provided novel insights and new avenues for future studies.
Overall, the experiments are well designed and results are well presented.

I have only a few comments:

-In Fig5A. I wonder whether the ATRX localization in matPCH is changed in either HP1 or Suv39h2 mutants since ATRX is part of the ADD complex.

-Is the maternal Daxx phenotype related to H3.3K36-H4K16ac and H1 mechanism from Lin et al., 2013 Development?

-The comparison of different stages of oocytes (GV oocytes in Daxx and MII oocytes in Rnf/Ring) seems to me not a fair comparison. Do the authors have additional data of the ATRX mutants to support the minimal transcriptome changes in the Daxx mutant oocytes/embryos?

-Typos of Page4 line 30:
EGFP-tagged H3.2 (not 3) and mCherry-tagged H3.3 (not 2)?
What are Fully-Grown metaphase II (M-II) oocytes? Usually, we call fully-grown GV oocytes and matured MII oocytes.

-Page 6 line30: Provide evidence of H3.3 interact with Cbx2.

-Fig. 6B is missing

-Figure S5H is not very clear for me.

- Rearrange the order of text and figures page 8 line 25-36 and page 10 line18-25
FigS5 showed up in the text before FigS4F, G;
introduce the result of pat-PCH before H2AK119;
repeated sentences of page10 line18-25 and page8 25-36

1st Revision - authors' response 7 February 2020

The point-by-point response to the reviewers' comments are given in blue.

Referee #1:

The manuscript "SUMO-PRC1 controls genome integrity by DAXX-mediated H3.3 incorporation in embryonic heterochromatin" by Liu et al. describes complex mechanism of DAXX-mediated control of H3.3 deposition into the pericentromeric heterochromatin (PCH) in paternal pronuclei of mouse zygotes. The authors have demonstrated that the process is tightly controlled by sumolyation of CBX2 (the component of Polycomb Repressive Complex, PRC1), catalysed by SUMO2. The sumolyated CBX2, in turn, directly interacts with DAXX and facilitates the deposition of H3.3 into the target genomic loci. The presence of H3.3-containing nucleosomes directly provides chromosomal stability during the first embryonic cell division, thus imposing biological impact on early embryonic development. The provided experimental data demonstrate the dual role of DAXX on genome activity: it's deficiency results in reduced transcription of major satellite repeats, but up-regulates the expression of some genes, related to development and differentiation.

The manuscript is clearly structured and well written, allowing the reader to understand rather complex interactions between the multiple components, regulating and mediating DAXX activity. The experimental data and the logical rationale behind the experiments are solid, as well as the statistical evaluation of the data.
We thank the reviewer for the strong appreciation for the manuscript and the constructive comments.

There are a few minor points which still should be addressed:

Q: Figure 1A. The zygote in the middle panel looks more like PN1, rather than PN2/3 - which means that the DNA replication has not even started yet. Also the presence of H3.2-GFP signal inside the pronuclei does not automatically imply the incorporation of the histone into the chromatin.
A: Thank you for pointing this out. We acknowledge the ambiguity in specifying the pronuclear stage of the original image. To resolve this, we replaced the panel with a new image, showing H3.3-mCherry and H3.2- EGFP fluorescence in both pronuclei of the embryo at a clear PN3 stage. In addition, we included to the manuscript, as a supplementary document, the live imaging movies from which the original still images were taken.

Q: Figure 6B is missing.
A: Our apologies for the omission of panel 6B which occurred during the preparation of the TIFF files. The image is now included in the final figure.

Q: In figures descriptions - what is the difference between "WT" and "Daxx". I suppose that both refer to WT embryos.
A: For the data presented within a given figure, we used the term Daxxm+z+ when the embryos where obtained from females which were littermates of the females that we used to obtain Daxxm-z+ or Daxxm-z-embryos.
We referred to WT when the mothers were either of C57Bl/6J origin and/or obtained from the Daxx (on C57Bl/6J background) or Ring1 Rnf2 (largely on the C57Bl/6J background) breeding colonies.

Q: It is curious, why the levels of Daxx mRNA are also strongly reduced in Daxx m-z- embryos? There should be only exon 2 missing, but the rest of mRNA could still be mappable?
A: We thank the reviewer for this interesting point. It inspired us to analyze the read counts corresponding to the Daxx transcript in oocytes and 2-cell embryos in more detail. These results are now incorporated in Figure EV5B and in the main text of the paper (at the top of the last results' paragraph before the discussion section) as follows:
"Compared to control oocytes, Daxx transcript levels were over 10-fold reduced in Daxx mutant oocytes, with >65% of residual transcripts lacking the floxed exon 3, showing an efficient depletion of Daxx expression. In contrast, Daxx mRNA levels were only <2 fold reduced in maternally deficient 2-cell embryos, with >65% being full length, indicating early and potent transcriptional activation of the paternal allele in Daxxm-z+ embryos (Fig EV5B), which relates to immuno-detectable DAXX protein from the four cell stage onwards (EV4A, B)."

Referee #2:

This interesting paper addresses how the chromatin landscape is established in the pericentric heterochromatin (PCH) of paternal chromosomes in mouse pre-implantion embryos. Whereas the female genome is maintained as chromatin from oocyte to zygote, the paternal genome in sperm is largely devoid of histones and instead is packaged using protamines and on fertilisation the paternal chromatin needs to be re-established. Whereas the maternal PCH is marked by histone H3K9me and HP1, the paternal PCH contains the replacement histone variant H3.3. This research group, in previous publications, have determined many of the steps in this process and this report concentrates on the role of the histone H3.3 chaperone Daxx. Here they show the importance of paternal genome-specific sumoylation with SUMO2/3 of CBX2, a component of the polycomb complex PRC1. This drives recruitment of Daxx via its sumo interacting domains which in turn leads to H3.3 deposition. This process of establishing chromatin is needed to maintain genomic stability and in its absence there is decompaction of PCH, chromosome breakage and mis-segregation. A surprising result given that Polycomb represses transcription of PCH was the finding that knocking out Daxx itself led to substantial repression of transcription. This leads them to conclude that though Daxx works in the same pathway as Polycomb their opposing roles must be involved in balancing expression.
This is a very comprehensive study, the experiments are well designed and executed and data is clear and persuasive. One criticism is that the relevance of this work in the field is skated over. It is also very densely written and this contributes to a rather hard read. One area not explored is whether ATRX, a chromatin remodeller and a partner of Daxx, plays a role in establishing the paternal PCH chromatin. However, this is probably beyond the scope of this report and could be a focus of future research.
We thank the reviewer for the very supporting feedback to our manuscript. We hope that the readers will recognize and appreciate the relevance of our comprehensive mechanistic study as well. As indicated by the reviewer, we consider that studying the role of ATRX in pre-implantation development goes beyond the current manuscript.

Minor points:

Q: In Fig 6B data is missing.
A: Our apologies for the omission of panel 6B which occurred during the preparation of the TIFF files. The image is now included in the final figure.

Q: There are no legends associated with the movies.
A: Our apologies for the omission of these legends. The legends of all supplementary movies are now described in the Appendix document.

Q: Figure 6H the reduction in H3.3-EGFP fluorescent signal is said to be "major" but I think this is rather subtle and it is difficult to define the nucleolar body especially in the Daxxm-z+. It might help if a Dapi stain was included.

A: We acknowledge that the still images of the live-imaging movies do not convey so well the change in H3.3 enrichment around the nucleolar precursor bodies in paternal pronuclei that we had observed in the live-imaging movies. To further support our conclusion, we now provide movies from which the still images were taken (Appendix movies S6 – S8).

Q: Where is the ontology analysis mentioned on p13 line3?
A: To clarify this point, we added a reference to Table EV2 into the text. In the original text, we referred to this table in the subsequent paragraph.

Q: The data in Fig 7J is presented in a rather odd way - why not present in the same way as Figs 3F-H?
A: We prefer to present the data in the format of Fig 7J since it allows a direct visualization of the statistical testing that we performed.

Referee #3:

In this manuscript, Liu et al. investigated the maternal function of Daxx, an H3.3 chaperone, on the formation of pericentromeric heterochromatin (PCH). In addition to their previous paper (Tardat et al., 2015 Mol Cell) which built up a PRC1-Cbx2 pathway in paternal PCH and Suv39h2-HP1 in the maternal PCH, the authors provided new pieces of evidence of their interaction with Daxx/ATRX-H3.3 complex via SUMOylating. The differences in recruitment of Daxx and ATRX between maternal PCH and Paternal PCH also provided novel insights and new avenues for future studies. Overall, the experiments are well designed and results are well presented.
We thank the reviewer for her/his appreciation of our research and the valuable constructive feedback that helped us to further improve our manuscript.

I have only a few comments:

Q: In Fig5A. I wonder whether the ATRX localization in matPCH is changed in either HP1 or Suv39h2 mutants since ATRX is part of the ADD complex.
A: This is an interesting question. Yes, since ATRX is recruited to canonical heterochromatin via its interaction with H3K9 methylation and HP1 proteins, its localization is indeed changed in the respective mutants. Instead, the protein now colocalizes with DAXX at maternal PCH in a “pearls on a string” configuration as observed at paternal PCH in wild-type embryos. We incorporated this data in a revised panel for Figure 5A in which we omitted the co-staining of DAXX with Hp1β and instead show DAXX and ATRX localization. In Figure 5B, we provide the quantification of the new data.

Q: Is the maternal Daxx phenotype related to H3.3K36-H4K16ac and H1 mechanism from Lin et al., 2013 Development?
A: We thank the reviewer for pointing out this interesting paper. We have not analyzed H4K16ac and H1 levels in Daxx deficient embryos. We therefore discuss this paper as follows in the discussion of the revised manuscript:
“Interestingly, morpholino-mediated depletion of total H3.3 protein showed that H3.3 sustains proper chromosome segregation throughout pre-implantation development. Moreover, H3.3 maintains a decondensed chromatin state, in part by directing MOF-mediated H4K16 acetylation and counteracting H1 incorporation, and which is required for development beyond the morula stage (Lin et al., 2013). To understand the possibly multiple mechanisms underlying the developmental and cytokinesis defects observed in Daxxm-z- embryos, it will thus be important to assess the impact of Daxx deficiency on general mitotic chromatin structure as well as on gene expression during late pre-implantation development.”

Q: The comparison of different stages of oocytes (GV oocytes in Daxx and MII oocytes in Rnf/Ring) seems to me not a fair comparison. Do the authors have additional data of the ATRX mutants to support the minimal transcriptome changes in the Daxx mutant oocytes/embryos?
A: Unfortunately, we do not have the requested data available, which is due to a currently small mouse colony. We do acknowledge that the comparison of MII oocytes and 2-cell embryos (Daxx control and mutants) versus GV oocytes (Ring1/Rnf2) is not entirely spotless. Yet, technically speaking, the comparisons were done at the "relative level" between mutants and controls at a given developmental stage and genotype. Such approach cancels out any differences that are due to differences in developmental stages between conditions.
By relating expression changes to H3K27me3 levels, an obvious orthogonal data set, we noticed a significant enrichment for H3K27me3 occupancy among genes upregulated in Daxx deficient MII oocytes and 2-cell embryos as also observed for genes upregulated in Ring1/Rnf2 deficient GV oocytes (Figures 7G-7I). In Figure 7J, the direct comparison between expression differences confirms that similar sets of genes are co-regulated by DAXX as well as PRC1.
Su et al. (Developmental Biology, 2007) described that the transition from GV to MII stages is characterized by a selective destruction of transcripts that are associated with meiotic arrest at the GV-stage and the progression of oocyte maturation. In the Daxx mutant MII oocytes, however, we observed a more pronounced up-regulation than down-regulation of transcripts, many of which are associated with more developmental embryonic functions rather than oxidative phosphorylation, energy production, and protein synthesis and metabolism. We therefore interpret that the comparison between the two mutant conditions is not confounded by the inherent differences between the two developmental stages studied.

Q: Typos of Page 4 line 30:
EGFP-tagged H3.2 (not 3) and mCherry-tagged H3.3 (not 2)?
A: Thank you for noticing this mistake. We corrected it.

Q: What are Fully-Grown metaphase II (M-II) oocytes? Usually, we call fully-grown GV oocytes and matured MII oocytes.
A: We agree to the mentioned nomenclature and removed the word "fully-grown" in the respective sentence.

Q: Page 6 line30: Provide evidence of H3.3 interact with Cbx2.
A: Original co-immunoprecipitation experiments revealed pulldown of H3.3 with Flag-tagged CBX2. However, given the present focus on the interaction between CBX2 and DAXX, we decided to exclude this data from the current manuscript. Apologies for the inconvenience and thank you for pointing it out. We edited the text in the revised manuscript as follows:
"We found that CBX23×Flag co-immunoprecipitated with DAXXMycHis and endogenous RNF2, even in the presence of benzonase, excluding a role for RNA or chromatin in these interactions (Fig 2D). CBX23×Flag also immunoprecipitated H3K27me3, likely reflecting the interaction with the chromodomain of CBX2 as demonstrated previously (Tardat et al., 2015)."

Q: Fig. 6B is missing
A: Our apologies for the omission of panel 6B which occurred during the preparation of the TIFF files. The image is now included in the final figure.

Q: Figure S5H is not very clear for me.
A: We acknowledge that the figure was not so easy to understand and therefore decided to remove the panel from the figure. We think that the text in the manuscript is sufficiently clear, without showing the actual data.

Q: Rearrange the order of text and figures page 8 line 25-36 and page 10 line18-25 FigS5 showed up in the text before FigS4F, G; introduce the result of pat-PCH before H2AK119; repeated sentences of page10 line18-25 and page8 25-36
A: To accommodate the reviewer's suggestion, we reorganized the figures in such a way that the individual panels are presented and discussed in the "chronological" order of the text flow in the manuscript.

2nd Editorial Decision 9 March 2020

Thank you for submitting your revised manuscript for our consideration, it has now been seen once more by the original referees (see comments below). I am pleased to say that the referees find that their concerns have been satisfactorily addressed and now support publication. I would therefore like to ask you to now address some editorial issues that are listed in detail below in a final revised version. Please make any changes to the manuscript text in the attached document only using the "track changes" option. Once these minor issues are resolved, we will be happy to formally accept the manuscript for publication.

REFEREE REPORTS

Referee #1:

The author addressed and answered all questions. They added new (and clearer) data to their manuscript. Given this improvement I would now recommend publication.

Referee #2:

The authors have satisfactorily addressed the points raised.

Referee #3:

The authors have provided a revised manuscript that addressed the reviewer's comments and concerns. The reviewer is supportive of publication of this manuscript in EMBO J.

2nd Revision - authors' response 9 April 2020

The authors performed all minor editorial changes.

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: Antoine Peters
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2019-103697

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 $\chi 2$ 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.

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

http://datadryad.org

http://figshare.com

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

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

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

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 majorly determined by availability of embryonic samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	All sample sizes of mouse embryos are indicated in figures or their legends.
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.	Samples were allocated to groups on the basis of their genotype. Randomization was not applied.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was not applied.
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.	Quantification of imaging data.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Samples were grouped based on genotypes in a non-blinded manner. Immunofluorescence data was quantified using the same procedure, irrespective of genotype.
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. We used the SigmaStat 3.5 program for testing statistical significance. The program also indicates whether requirements of normality and equal variance are met.
Is there an estimate of variation within each group of data?	Yes, please see above.

Is the variance similar between the groups that are being statistically compared?	Yes, please see above.

C- Reagents

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).	Appropriate information is described in the methods section
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Source is given.

* 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.	See materials and methods section
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	See materials and methods section
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.	I confirm compliance.

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	The datasets produced in this study are available in the following databases: RNA-Seq data: Gene Expression Omnibus GSE137030 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137030) RNA-Seq data: Gene Expression Omnibus GSE133442 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133442) ChIP-Seq data: Gene Expression Omnibus GSE107348 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107348).
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.	The method for quantification of signal intensity in different nuclear compartments of zygotes is described in the method section.

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