EMBO reports

Tet enzymes are essential for early embryogenesis and completion of embryonic genome activation

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Transaction Report:

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Dear Mark,

Thank you for the submission of your research manuscript to our journal. You report that Tet enzymes are essential for early mouse development and genome activation. Your study had been reviewed and revised for another journal that could not offer publication. We note that referee 1 supported publication after revision, while referee 2 remained concerned that the study remained descriptive without offering mechanistic insight into the catalytic and non-catalytic roles of TET2 in early development and gene expression. Referee 3 remained concerned regarding the use of a truncated form of Tet2 instead of a catalytically inactive full-length Tet2 in the rescue experiments. We also note that referee 1 and 3 considered the finding that Tet enzymes play a role in genome activation and early mouse development of interest to the community and given that EMBO reports does not require a full mechanistic understanding, we considered the study a potentially good fit for publication in EMBO reports.

We have therefore discussed the remaining concerns further with an expert in the field. The expert agreed that the data on the Tet2 truncation construct should remain part of the study but that it needs to be complemented with an extended discussion of the value of the chosen truncation construct and potential alternative scenarios and limitations, such as the disruption of protein-protein interactions. Furthermore, we asked the advisor to comment on the specificity of the morpholino-based knockdown approach, which was one of the concerns raised during review. The expert advisor noted also that the phenotype you observe upon MO-induced knockdown of Tet enzymes in oocytes differs from that reported for germline-deleted Tet2 mice, which are fertile. However, the advisor also acknowledged the different approaches and supported publication, if the contradiction between the fecundity of germline-deleted Tet2 KO mice and your observations with morpholino-injected oocytes are explicitly acknowledged and thoroughly discussed.

Based on this evaluation and the advisor's feedback, we have decided to proceed with the publication of your manuscript, pending that the above-mentioned issues are appropriately discussed.

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- Please rewrite the abstract in present tense and shorten it to 175 words. I also suggest to mention the morpholino-based approach in the abstract, as it currently seems to imply the analysis of KO embryos.

- Supplementary figure S12: Please define the central band, boxes and whiskers of the boxplot.

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- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points and do not calculate statistics.
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- Whole genome bisulfite-sequencing

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Data availability

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- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
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Kind regards,
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Martina Rembold, PhD
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Dear Mark,

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Based on this evaluation and the advisor's feedback, we have decided to proceed with the publication of your manuscript, pending that the above-mentioned issues are appropriately discussed.

We thank the expert for his fair assessment of our work. We now acknowledge and discuss the contradiction of our morpholino-based model to the KO model in greater detail in several parts of our revised manuscript and include an extended discussion of the truncated Tet2 construct applied in our study. Here is a summary of the parts discussing these aspects:

Page 4: “Observed Tet-KO phenotypes are obvious during postimplantation development, with preimplantation embryos showing mild transcriptomic changes or developmental impairment in different contexts. Here, heterozygous Tet3-KO mice displayed neonatal abnormalities, and Tet1-/- and Tet2-/- single KO-embryos developed with postnatal malignancies (Dawlaty et al, 2011; Gu et al., 2011; Ko et al, 2011; Li et al, 2011).”

Page 6: “Our observation using a morpholino-based knockdown approach in fully grown oocytes stands in contrast to phenotypes observed upon germline deletion of all three Tet-enzymes, where phenotypes are emerging in early postimplantation development (Dai et al., 2016). Both approaches have advantages and limitations and target different questions. The Tet-TKO approach diminishes Tet

proteins during the growth phase of the oocyte – a phase where massive DNA remethylation is still occurring and compensation mechanisms can intervene, while the morpholino-based knockdown approach is effective in fully grown and transcriptionally silent oocytes, minimizing compensatory effects and only mimicking loss of Tet function specifically during the oocyte-to-embryo transition. Moreover, the Tet-TKO and most single Tet-KO models aimed to create genomic deletions of the catalytical domain of Tet enzymes leaving the expression of a truncated product (Dai et al., 2016; Gu et al., 2011; Ko et al., 2011; Zhang et al, 2013), while the morpholino-based KD diminishes the expression of the complete gene product. Albeit Cre/lox mediated KO approaches are highly specific and KD approaches bare possibilities of unspecific off-target effects, KO strategies can lead to unspecific phenotypes due to non-sense mediated decay of truncation constructs (El-Brolosy et al., 2019; Ma et al., 2019; Wilkinson, 2019), whereas in MO-based KD approaches the full-length RNA is still available but blocked for translation.

Page 8: "These observations for Tet2-KD were unexpected and are in contrast to published Tet2-KO models, similarly to the Tet-TKD/Tet-TKO discrepancy (Ko et al., 2011; Li et al., 2011)."

Page 9: "Thus, our rescue experiments validated the specific phenotype we observed in Tet2-KD embryos, which differs from the reported phenotype in Tet2-KO mice, which develop to term and are fertile (Ko et al., 2011; Li et al., 2011).

In summary, our analysis of Tet-KD experiments revealed a striking early developmental arrest of Tet-TKD embryos primarily at the 2-cell stage upon acute depletion of Tet enzymes in the oocyte-to-embryo transition, with the most severe phenotype linked to Tet2."

Page 18: "We designed a rescue experiment for Tet2 using a truncated Tet2 mRNA mimicking the catalytical KO of Tet2 in previous germline Tet-TKO study (Dai et al., 2016)(Appendix Fig. 4B and D). While truncated expression products of genes can mediate non-sense mediated decay, as discussed as a complication in KO approaches (Wilkinson, Nature 2019), or lead to disruptions of protein-protein interactions, a shorter isoform of Tet2 (missing the catalytical domain) is specifically expressed in human oocytes and early human cleavage stage embryos, suggesting an important non-catalytic function of the C-terminal part of Tet2 (Hendrickson et al., 2017)."

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We reformatted the text to fit to the "report" style (combined "Results and Discussion")

- Please rewrite the abstract in present tense and shorten it to 175 words. I also suggest to mention the morpholino-based approach in the abstract, as it currently seems to imply the analysis of KO embryos.

We shortened, rewrote in present tense, and now mention the morpholino-based approach and thank you for the suggestion.

- Supplementary figure S12: Please define the central band, boxes and whiskers of the boxplot.

We now define this in the figure legend.

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- 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} 2, use scatter blots showing the individual data points and do not calculate statistics.
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.

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2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
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Not applicable to our study.

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Title: Tet enzymes are essential for early embryogenesis and completion of embryonic genome activation
Author(s): Julia Arand, H. Rosaria Chiang, David Martin, Michael Snyder, Julien Sage, Renee Reijo Pera, and Mark Wossidlo

Dear Mark,

Thank you for your patience while we have editorially checked and reviewed your revised manuscript and point-by-point response. As you know, we perform a number of quality checks on manuscript figures and text, and there are some minor issues that still need your attention.

I am thus writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

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Thus, in the text Cotto et al needs to be cited (preprint: Cotto et al, 2021) and in the reference list you need to add the doi and the identifier [PREPRINT].

- Please add callouts to the panels of Appendix Fig S9.

- Please upload Tables Tables EV1, EV2, EV4, EV7, and EV8 as Dataset EV files. The nomenclature is Dataset EV# and the legend can stay as is, i.e., part of the Dataset .xls file.

- Please change the names of Appendix Figs S1 and S4 to Appendix table S1 and S2 and please also update the callouts in the text accordingly. I also noticed a type on the legend of Appendix Fig. S9 ("each dots represents one ..." should be "each dot represents....")

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The authors performed the requested editorial changes.

Dr. Mark Wossidlo
Medical University of Vienna
Vienna A-1090
Austria

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

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- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
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- ➔ 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.).
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  - 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.

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

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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.	Oocytes from several female mice per experiment were pooled and randomly picked for each condition.
For animal studies, include a statement about randomization even if no randomization was used.	Oocytes from several female mice per experiment were pooled and randomly picked for each condition.
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.	Blinding was not applied in the experiments
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding was not applied in the experiments
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.	Normal distribution was check with statistical test (Shapiro-Wilk) and/or visually checked with appropiate plots (Box-Plot/QQ-plot/Frequency distribution) for data sets analyzed with ANOVA or paired t-tests.
Is there an estimate of variation within each group of data?	Standard deviations are indicated in all analyzed datasets.

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
http://www.consort-statement.org/checklists/view/32-consort/66-title

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

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Not always - test was choosen appropiately

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).	We provide this information in the method 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.	NA

* 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.	We provide this information in the method section.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All mouse experiments were carried out in accordance to the Stanford University Administrative Panel on Laboratory Animal Care and the Medical University in agreement with the authorizing committees.
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.	We follow the ARRIVE guidelines.

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	Data availability section is included in the manuscript: The datasets produced in this study are available in the following databases: RNA-seq data: Gene Expression Omnibus GSE57063 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57063) Whole genome bisulfite-sequencing: Gene Expression Omnibus GSE156006 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE156006)
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).	Datasets were deposited to Gene Expression Omnibus and selected critical analyses are included in EV Tables. We furthermore provide EV Movies.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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