EMBO reports

Manuscript EMBO-2015-41439

The Tau/A152T mutation a risk factor for frontotemporal spectrum disorders leads to NR2B-mediated excitotoxicity

Jochen Decker, Lars Krüger, Astrid Sydow, Frank JA Dennissen, Zuzana Siskova, Eckhard Mandelkow, Eva-Maria Mandelkow

Corresponding author: Eva-Maria Mandelkow, Center for Neurodegenerative Diseases

Review timeline:

Submission to The EMBO Journal	21 July 2015
Editorial Decision:	14 September 2015
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Editorial Decision:	06 October 2015
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Revision received:	27 January 2016
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Editor: Esther Schnapp

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.)

DECISION FROM THE EMBO JOURNAL

1st Editorial Decision 14 September 2015

Thank you for submitting your manuscript to The EMBO Journal. I am very sorry for the delay in getting back to you with a decision, but I have now received the comments back from the two referees who reviewed your manuscript. I am afraid that the overall opinion is not very positive.

The referees appreciate that the analysis is well done, but they are also not convinced that the advance and insight provided is sufficient to consider publication here. In particular referee #2 finds that we gain too limited novel mechanistic insight into how tau promotes neurodegeneration over other models and why the described variant is more toxic that wildtype tau. Given the referee comments and as we require strong support from referees for consideration here I can unfortunately not offer to consider publication here.

Given the interest in the topic I have taken the opportunity to discuss this manuscript and the back-to-back submission from The Mucke lab with my colleague Esther Schnapp from EMBO Reports. EMBO Reports is interested in considering both manuscripts for publication in EMBO Reports and will work with the two referee reports on hand. They don't need any further mechanistic insight nor further comparison to other mouse models. They would like some clarification on the neuronal loss issue as raised by referee #2. If you are interested in transferring to EMBO Reports, I would suggest that you contact Esther Schnapp at esther.schnapp@embo.org to discuss this option further with her.

For the EMBO Journal. I am sorry that I can't be more positive on this occasion, but I hope that you will consider the EMBO Reports option.

REFEREE REPORTS

Referee #1:

This is a very thorough characterization of a tau 152/T mutation that links overexpression of this to NR2B mediated excitoxicity. As this mutations appears to be a risk factor for FTD/PSP/.CBD and AD understanding how it is similar and different to other tau mutants associated with FTD is important. It is also relatively unique in that this mutant is in the N-terminal domain away from the MT binding site. Overall I think this is an important addition to the field that characterizes the new line of mice and shows interesting linkage to glutamate excitotoxicity. A few concerns are listed below.

Though the manuscript itself is very internally consistent, the major limitation is that the A152T mutant is not only compare to a non-TG not a wt human tau transgenic control. Although the group has studies other models and claims differences between those tau models and this one, I think one has to temper the claims unless some side by side comparisons to the wild type human in the same ROSA locus are made. If this cannot be achieved experimentally then I think the discussion just needs to be a little more cautious.

It would be nice to know the overexpression at the transcript level relative to the overexpression at the protein level.

A transgene is not physiologic really by definition unless it is a knockin. Thus the statement: This results in physiological expression levels in the hippocampus of heterozygous (+/-) and homozygous (+/+) hTauAT mice at an age of 14 month (Fig 1B)." should be amended.

This sentence is not logical: "Gallyas silver staining (Fig 2B) and sarcosyl extraction (Fig 2C) indicate a strong co-aggregation of endogenous mouse Tau and exogenous human Tau)" Gallyas silver staining should be uncoupled from the sarcosyl extraction.

Are there any higher MW tau species on the blots (the ones that ae shown are truncated).

Referee #2:

This is an extremely well-assembled and well-performed study showing the consequences of transgenic over-expression of the A152T tau variant in mice. The authors use electrophysiology to define a possible mechanism of excitotoxicity caused by this tau variant through NDMARs. The evaluation of this particular mutant in mice is new, and the studies are well-done. But it is unclear how this model provides new insights into the mechanism of tau-mediated neurodegeneration over other existing models. It is also unclear how this variant is more toxic than wildtype tau since the A152T is primarily a risk variant of disease. This would suggest that there must be a trigger that makes this tau more likely to become pathogenic and that is not determined by these studies.

Overall, if the authors could show that this variant does cause progressive neuronal loss to a greater extent than other variants or could provide a mechanism for how A152T becomes more toxic or pathogenic than wildtype to a stimulus, then there would be more enthusiasm. The excitotoxicity seen in slice cultures would suggest that neuronal loss is occurring progressively in these mice, but this data is not shown. Was stereology performed? Figure 2 suggests that neurons are degenerating, but to what extent and where is the degeneration occurring, if at all? In addition, if the authors could provide a mechanism for how tau is enhancing pre-synaptic transmitter release, that would also elevate this work.

So overall, this is an extremely well done paper. but the mechanistic insights about the differences between wildtype and this variant, or how tau enhances pre-synpatic activity are not clear. Also the

lack of progressive neurodegeneration makes this a model of tau over-expression with limited utility relative to what is already available.

EMBO REPORTS

1st Editorial Decision 06 October 2015

Thank you for the transfer of your research manuscript with referee comments to EMBO reports. As discussed, we can consider a revised manuscript for publication, and additional insight into mechanism will not be required for EMBO reports.

However, please address the referee concerns to the best of your abilities in a complete point-by-point response and also provide data on progressive neuronal loss in your tau mutant mice.

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. Given your 8 main figures, we will publish your manuscript as a regular research article. For a normal article there are no length limitations, but the results and discussion sections must be separate and the entire materials and methods included in the main manuscript file. Please note that supplementary data have changed into expanded view (EV) figures and EV tables that are embedded in the main manuscript online. At the moment, our publisher can only process 5 EV figures per manuscript. If you have additional supplementary figures please include these in the Appendix file (see our guide to authors for more information). The figure legends for EV figures need to be added at the end of the main manuscript text, legends for Appendix figures need to be in the Appendix. Please upload EV figures as individual files.

Please also change the reference style to the numbered EMBO reports style.

Regarding statistics, please remember to specify "n", bars and error bars and tests used to calculate p-values in each respective figure legend.

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.

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

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1st Revision - authors' response 15 December 2015

Referee #1:

Point 1
"Though the manuscript itself is very internally consistent, the major limitation is that the A152T mutant is not only compare to a non-TG not a wt human tau transgenic control. Although the group has studies other models and claims differences between those tau models and this one, I think one has to temper the claims unless some side by side comparisons to the wild type human in the same ROSA locus are made. If this cannot be achieved experimentally then I think the discussion just needs to be a little more cautious."

We revised and toned down parts of our manuscript where claims about direct comparisons between the A152T Tau mutation and other FTDP-related mutations were made.

Discussion, §4, page 9: " It is notable that different Tau mutations may have opposite consequences on synaptic transmission in mice: Pro-aggregant mutations in Tau's repeat domain (Δ280) lead to reduced transmission in the mossy fiber tract (Van der Jeugd et al., 2012, Sydow et al., 2011, Decker et al., 2015) whereas the amino-terminal mutation A152T, described here, enhances transmission in the same pathway. By analogy, there can be opposite effects in terms of calcium levels, which are reduced in neurons expressing $Tau^{RD\Delta K280}$ (Messing et al., 2013, Decker et al., 2015) and increased when A152T Tau is expressed."

Point 2
"It would be nice to know the overexpression at the transcript level relative to the overexpression at the protein level."

The data on the transcript level had been available but were not included in the previous version. Now we included them into Fig. 1D and compare them with the protein level, as follows: "The $hTau^{AT}$ mRNA levels in transgenic heterozygous (1.00 ± 0.35 A.U.) and homozygous (2.19 ± 0.27 A.U.) mice (16 month) were in good agreement with the hTau/mTau protein expression ratios in hippocampi from transgenic animals (compare Fig 1C to Fig 1D)." (Results, §1, page 4)

Point 3
"A transgene is not physiologic really by definition unless it is a knockin. Thus the statement: This results in physiological expression levels in the hippocampus of heterozygous (+/-) and homozygous (+/+) hTauAT mice at an age of 14 month (Fig 1B)." should be amended."

We agree that a transgenic line is per se not physiological and therefore exchanged the term as follows:
"This results in moderate overexpression levels in the hippocampus of heterozygous (+/-) and homozygous (+/+) $hTau^{AT}$ mice at an age of 14 month (Fig 1B)." (Results, §1, page 4)

Point 4
"This sentence is not logical: "Gallyas silver staining (Fig 2B) and sarcosyl extraction (Fig 2C) indicate a strong co-aggregation of endogenous mouse Tau and exogenous human Tau)" Gallyas silver staining should be uncoupled from the sarcosyl extraction."

The sentence has been changed as follows:
"Using two complementary techniques, Gallyas silver staining (Fig 2B) and sarcosyl-extraction (Fig 2C) we could identify aggregated Tau species in 14 months old mice. Interestingly, both human and mouse Tau was found in the sarcosyl-insoluble fraction indicating strong co-aggregation (Fig 2C)." (Results, §1, page 4)

Point 5
"Are there any higher MW tau species on the blots (the ones that are shown are truncated)."

We do not see higher MW Tau bands than those shown in the figures 1 and 2.

Referee #2:

"The excitotoxicity seen in slice cultures would suggest that neuronal loss is occurring progressively in these mice, but this data is not shown. Was stereology performed? Figure 2 suggests that neurons are degenerating, but to what extent and where is the degeneration occurring, if at all?"

The reviewer correctly anticipated that there would be neuronal loss in aged $hTau^{AT}$ mice. Quantitative real-time PCR analysis for the neuronal marker NeuN in hippocampi of aged $hTau^{AT}$ mice reveal a reduction of neurons of ~30% (Fig EV2C) which is well comparable to NeuN-data in slice cultures (Fig 5C, D). We now complemented the slice culture data on neuronal loss by two new time points - an early one at DIV 10 and a late one at DIV 60. When comparing neuronal numbers at the three different time points between control and $hTau^{AT}$ slices, we can demonstrate a progressive

neuronal loss due to hTau^{AT} transgene expression in the slice culture model. Wording was changed as follows:

"We further analyzed neurons in slices at an earlier (DIV 10; Fig EV2A) and a later time point (DIV 60; Fig EV2B). At DIV 10 no significant cell loss was detected in areas DG, CA1 and CA3. In contrast, at DIV 60 neuronal loss was even more pronounced when compared to values observed at DIV 30." Results §11 page 6.

To further strengthen the aspect by *in vivo* data we show a hippocampal section after immunohistochemistry for NeuN of control and aged hTau^{AT} mice in the new version of our manuscript (Fig. 5E). In agreement with the observations in slice cultures, neuronal cell loss was prominent in the pyramidal layer of area CA3 in 12 months old heterozygous hTau^{AT} mice (Fig 5E). To test if neuronal cell death occurs progressively in our Tau mice, we analyzed NeuN protein levels by western blot analysis in hippocampi of 12 and 18 months old animals (Fig 5F-G). NeuN expression was reduced by ~10% in 12 months old hTau^{AT} mice and neuronal loss became more prominent to ~35% in 18 months old hTau^{AT} mice when compared to controls (Fig 5F, G) indicating that cell loss occurs indeed progressively in our hTau^{AT} mice. Wording was changed as follows: "These findings in slice cultures were supported by the observation of neuronal cell loss in the hippocampus of aged hTau^{AT} mice. Similar to what we have seen in slice cultures, neuronal cell loss was prominent in the pyramidal layer of area CA3 in 12 old heterozygous hTau^{AT} mice (Fig 5E). Next we analyzed NeuN protein levels in hippocampi of 12 and 18 months old hTau^{AT} mice (Fig 5F-G). Here we found a progressive loss of NeuN expression which was reduced by ~10% already in 12 months old hTau^{AT} mice and neuronal loss increased to ~35% in 18 months old hTau40^{AT} mice when compared to control mice (Fig 5F, G)." Results §11 page 6.

Finally we described cellular and sub-cellular level degeneration in detail by electron microscopy in area CA3 of hTau^{AT} mice (Fig 2E-J).

2nd Editorial Decision 05 January 2016

Thank you for the submission of your revised manuscript to our journal. We have received the comments from the referees now and I am happy to tell you that both support its publication now.

Referee 2 suggests to move some of the data to the expanded view in order to increase the readability of the paper. If you find this suggestion useful, please make these changes. You can send us the modified files by email, and we will replace the current files for you. Also, the abstract needs to be written in present tense, and if you prefer, we can make these changes for you. We will also need to remove the list of abbreviations, as our research papers do not support this format, so please verify that the less common abbreviations are spelled out in the manuscript text.

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.

I look forward to seeing a final version of your manuscript as soon as possible and to accepting this nice paper.

REFEREE REPORTS

Referee #1:

I previously reviewed this paper along with a second related paper at EMBO Journal. This along with the other paper are very well done, but the novelty is not very high. The authors have done a nice job responding to this issue and I have no further comments that need to be addressed.

Referee #2:

The authors have adequately revised the manuscript. This is a very thorough study and a lot of data is presented. My only comment/suggestion is that readability would be increased by moving some data to the extended view versions, and reducing the amount of a data present within each figure. By keeping the main figures focused on the key points, the manuscript would be more readable.

2nd Revision - authors' response 27 January 2016

We have now modified the manuscript according to the referees' comments. In particular we added data on the progression of pathology. With these changes (marked in red in the resubmitted main text) we hope the paper becomes acceptable for publication.

3rd Editorial Decision 28 January 2016

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

Corresponding Author Name: Eva-Maria Mandelkow
Journal Submitted to: EMBO reports
Manuscript Number: EMBOR-2015-41439V2

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

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

http://datadryad.org

http://figshare.com

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

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

http://biomodels.net/

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

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?	We have chosen sample sizes in our study based on previous publications.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal studies on immunohistochemistry we have chosen at least three animals / group. For electrophysiological experiments we have chosen sample sizes of at least 5 animals / group. For RT-PCR and EM-studies we have used 4 animals / group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For Calcium imaging we used as exclusion criteria a) a stable base line for 30 seconds without rundown of >5%, b) a significant response after stimulation, c) an intact hippocampal morphology and d) comparable dye loading. During slice culture preparations mechanically damaged slices were excluded from 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.	Some experiments were performed in a blinded manner.
For animal studies, include a statement about randomization even if no randomization was used.	We have selected mice for experiments in a randamized fashion.
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.	The initial characterization of intracellular calicum levels (Figure 4A-C) in transgeni animals was performed blindly.
4.b. For animal studies, include a statement about blinding even if no blinding was done	For electrophysiology and electron microscopy of animals no blinding was used.
5. For every figure, are statistical tests justified as appropriate?	We have used the D'Agostino-Pearson omnibus test to test for normal distribution. Comparison of two groups was done by standard-t-test, and of more than two groups by ANOVA.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We have used the D'Agostino-Pearson omnibus test to test for normal distribution. Comparison of two groups was done by standard-t-test, and of more than two groups by ANOVA.
Is there an estimate of variation within each group of data?	In all our data presentations we show the SEM to display the amount of variation of our datasets.
Is the variance similar between the groups that are being statistically compared?	Yes, the variance is similar between the groups.

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).	ß-actin actin (Sigma; A3853); anti-neuronal nuclei (NeuN) antibody (Chemicon International, Temecula, CA, USA; Mab377), pan-Tau antibody K9JA (Dako, Hamburg, Germany, Nr. A0024, microtubule associated protein 2a/b (MAP2a/b) antibody (AP20; Sigma-Aldrich, Germany), human-specific TauY9 (Enzo Life Sciences, Germany; BML-TA3119-0100); PHF1 antibody for phosphorylated S396/404 Tau (gift from Dr. Peter Davies, Albert Einstein College, NY, USA); human-Tau specific antibody HT7 (Thermo Fisher Scientific, Waltham, MA, USA; MN1000), AT180 (Pierce, Rockford, USA; MN1040), AT8 (Thermo Scientific; MN1020), Alz-50 gift from Dr. P. Davies, Albert Einstein College, NY, USA). Rabbit polyclonal peptide antibody was generated against pT217 (1:1000, Biosource, Camarillo, CA, USA; Cat# 44-744)

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.	To achieve ubiquitous transgene expression in the brain at moderate levels the transgene (human full-length Tau carrying the mutation A152T) was inserted into the ROSA-locus of C57BL/6NTac embryo stem (ES) cells and injected C57BL/6NTac ES cells into BALB/c blastocysts (TACONIC, Germantown, NY). Injected blastocysts were transferred into the uterine horn of pseudopregnant NMR1 females. Chimerism was determined in chimeras by coat color contribution of ES cells to the BALB/c host (black/white). Highly chimeric mice were bred to C57BL/6 females. Germline transmission was identified by the presence of black offspring. The transgene expression is controlled by the neuron specific Thy1.2 promoter and occurs in the entire brain and spinal cord. The mouse strain was conceived on an identical C57BL/6N background. The present study presents data from heterozygous and homozygous hTauAT mice. Non-transgenic littermates were used as negative controls. All animals were housed and tested according to standards of the German Animal Welfare Act.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We comfirm compliance with the German Animal Welfare Act.

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 accession codes for deposited data. See author guidelines, under '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	NA
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. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. 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

23. 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