The EMBO Journal
Peer Review Process File - EMBO-2016-94892

Manuscript EMBO-2016-94892

**Natural underlying mtDNA heteroplasy as a potential source of intra-person hiPSC variability**

Ester Perales-Clemente, Alexandra N. Cook, Jared M. Evans, Samantha Roellinger, Frank Secreto, Valentina Emmanuele, Devin Oglesbee, Vamsi K. Mootha, Michio Hirano, Eric A. Schon, Andre Terzic and Timothy J. Nelson

*Corresponding author: Timothy J. Nelson, Mayo Clinic*

<table>
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<th>Review timeline:</th>
<th>Submission date: 30 May 2016</th>
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<td>Editorial Decision: 13 June 2016</td>
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<td>Revision received: 23 June 2016</td>
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<td>Accepted: 24 June 2016</td>
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*Editor: Andrea Leibfried*

**Transaction Report:**

(Note: This manuscript was reviewed at another journal before being transferred to The EMBO Journal. The original referee reports are not included in this Review Process file as they are not covered by the EMBO Press transparency policy.)

1st Editorial Decision 13 June 2016

Thank you for submitting your manuscript for consideration by the EMBO Journal. Your work as well as the point-by-point response to the referee reports you obtained previously at another journal have now been seen by two referees whose comments are enclosed.

As you will see, both referees think that despite the precedence for enrichment of mtDNA mutations in iPSCs your work could be published in The EMBO Journal, pending satisfactory minor revision. Importantly, you should revise your work to better emphasize and discuss the differences compared to the published study (see specific points from referee #1 and point 2 from referee #2). Furthermore, the title and figure 5 need to be changed to adequately reflect the conclusions from your work.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of both reviewers.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

-------------------------------------------

Referee #1:
In the manuscript by Perales-Clemente et al. the authors investigate the presence of potentially pathogenic mtDNA mutations in somatic cells that may become enriched in iPSC cells. They further show that these mutations may affect function of iPSC derived cardiomyocytes and propose screening of mtDNA as routine quality control for iPSCs. 

The study is well written and the message is clear. The experiments support the data well.

Unfortunately most of the data is not novel. The fact that iPSCs harbor mtDNA mutations has been shown before (Prigione et al. Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell-like metabolic reprogramming, Stem Cells, 2011) and the presence of these mutations in somatic cells and their enrichment in iPSCs was just shown by another group (Kang et al. Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs, Cell Stem Cell, 2016).

However, the current paper has some novelties that are of interest to the stem cell and mitochondria fields and the authors could emphasize and focus more on these aspects to make the paper stronger.

In the Kang paper the mutation levels correlate with donor age, while in the current paper no correlation is seen. This is an important aspect and the authors should expand this and discuss the discrepancy between the two studies. The authors should also refer to the Kang et al paper.

Another clear difference to the Kang paper is that in the current study all the mtDNA mutations present in the iPSCs were present also in the parental fibroblasts, whereas in the Kang et al. study majority of the mutations were novel and only some were shared between the iPSCs and the parental cells. This could be discussed as well.

Another interesting aspect that the authors raise is the co-segregation of certain mutations this aspect could be further expanded.

The point of the paper is that the novel unknown mutations may be pathogenic and lead to functional defects, yet in the functional study of the iPSC-derived cardiomyocytes the authors focus a lot on the effect of the m.3424A>G mutation to the cellular respiration. The effect of the novel mutations could be emphasized more and less emphasis put on the known disease-causing pathogenic mutation, which was known to be present in the cells. It would be nice to see data from healthy donor cells.

Minor points

The title says that mtDNA mutations are a source of intra-person human iPSC variability. This is a bit strong statement as it gives the idea that this is the source while the text it is stated as one potential source as it should be. While the mtDNA mutation are one of the sources, there definitely are other important sources as well, like mutations in the nuclear genome that have been shown to arise during reprogramming (several publications e.g. Mayshar et al, Cell Stem Cell, 2010, Hussein et al, Nature 2011).

Referee #2:

This manuscript performed mtDNA NGS on 84 human iPSC lines from 19 donors. The analyses found that many of the lines harbored various levels of mutations, which are likely attributable to heterogeneity of the original fibroblasts. The authors also showed that some of these mutations resulted in decreased oxidative respiration in differentiated progenies. Based on these findings, the authors proposed a flow chart to evaluate iPSC clones prior to clinical applications. I found the work important and interesting. It should be published in EMBO journal provided that the authors properly address the following issues.

1. The authors argued that the different degree of mtDNA mutations was a source of intra-person variability of the iPSC lines. This may be true, but requires more careful discussion. Clonal variation
of the iPSC lines includes differences in differentiation propensities and differences in functional performance after differentiation. As shown in figure 4, the authors did not observe the former. The authors did show that some mutations resulted in decreased oxidative respiration in cardiac myocytes, but whether this observation has any functional consequence remains unclear. Do these clones show any functional defects as cardiac myocytes? Without direct functional evidence, the title and the selection criteria shown in figure 5 would be too premature.

2. The authors should compare their results with those by Mitalipov and colleagues published earlier this year. Most notably, the two works differ sharply in age dependency of the mtDNA mutations. The authors provided nice figures in their responses to the reviewers in their previous submission, which I believe should be included in the manuscript.

Reviewers' comments:

Referee #1:

*In the manuscript by Perales-Clemente et al. the authors investigate the presence of potentially pathogenic mtDNA mutations in somatic cells that may become enriched in iPSCs. They further show that these mutations may affect function of iPSC derived cardiomyocytes and propose screening of mtDNA as routine quality control for iPSCs. The study is well written and the message is clear. The experiments support the data well. We appreciate the reviewer’s recognition of the quality of our work. We are encouraged by these comments.*

Unfortunately most of the data is not novel. The fact that iPSCs harbor mtDNA mutations has been shown before (Prigione et al. Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell-like metabolic reprogramming, Stem Cells, 2011) and the presence of these mutations in somatic cells and their enrichment in iPSCs was just shown by another group (Kang et al. Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs, Cell Stem Cell, 2016).

However, the current paper has some novelties that are of interest to the stem cell and mitochondria fields and the authors could emphasize and focus more on these aspects to make the paper stronger.

*In the Kang paper the mutation levels correlate with donor age, while in the current paper no correlation is seen. This is an important aspect and the authors should expand this and discuss the discrepancy between the two studies. The authors should also refer to the Kang et al. paper.*

This is indeed an emerging area of iPSC research that we have been part of with our paper in 2013. Additionally, there are inaccuracies in the few publications that will have a profoundly negative effect on the field if not addressed. We agree with reviewer #1’s comment about the importance of mutation level does not correlate with donor age in hiPSCs in our study. Following reviewer’s comments, we have cited and discussed Kang et al. paper in the page 14 of our manuscript. This is critically important.

“Our results show that low levels of heteroplasmic mtDNA variants are revealed in nuclear reprogramming regardless of donor’s age, as we found GPMs exhibiting heteroplasmic levels in excess of 10% in hiPSC clones-derived from individuals from one month of age to 44 years of age in control group, supporting the Universal Heteroplasm Theory as the process responsible for the differences exposed in mtDNA during nuclear reprogramming, rather than an accumulation of somatic mutations with age (Kang et al, 2016). Our findings demonstrate that hidden mtDNA heteroplasm can profoundly affect hiPSC function when differentiated into cardiac lineages and is consistent with emerging evidence that indicates a generalizable mechanism inherent to clonal expansion of hiPSC clones (Kang et al, 2016). Additionally, our data establishes that all ages of patients and independent of mitochondrial disease phenotype are vulnerable to mtDNA load. Conversely, low heteroplasmic hiPSC clones are commonly identified even from mitochondrial patients expected to have high heteroplasm based on clinical presentation.”
Another clear difference to the Kang paper is that in the current study all the mtDNA mutations present in the iPSCs were present also in the parental fibroblasts, whereas in the Kang et al. study majority of the mutations were novel and only some were shared between the iPSCs and the parental cells. This could be discussed as well.

This is indeed a critically important aspect of our study that we have inadequately discussed in the original version of the manuscript and now appropriately highlighted on pages 12-13. We appreciate the reviewers extremely helpful perspective to uncover this oversight.

"Previously, nuclear reprogramming was reported as the cause of new mtDNA mutations in hiPSCs (Prigione et al, 2011). Similar results were reported recently (Kang et al, 2016) showing low amount of shared mutation between hiPSC clones and the parental fibroblasts. However, our results obtained from mtDNA NGS analysis of 36 hiPSC clones and their parental dermal patient fibroblasts showed that all mtDNA variants present in the bioengineered stem cells were also present in the corresponding fibroblasts. mtDNA variants are exposed up to 100-fold during nuclear reprogramming (e.g. from 0.5% of m.9547G>A in the MitoA fibroblasts, up to 53% in MitoA clone 58). The extremely high depth of sequencing that was performed allowed us to separate the low level of heteroplasmy from the background noise. That could be a reason why low level of heteroplasmic mutations were not detected in previous studies in hiPSCs. Our results are in accordance with the Universal Heteroplasmy Theory (Payne et al, 2013) where very low-level of heteroplasmic point mutations (<1%) appears to be a universal finding among different healthy individuals.”

Another interesting aspect that the authors raise is the co-segregation of certain mutations this aspect could be further expanded.

Following reviewer’s recommendation, we have introduced the term co-segregation in the final version of the manuscript and expand this aspect on page 13.

“Moreover, mtDNA NGS analysis of 36 hiPSC clones derived from the three MELAS patients showed that some GPMs co-segregate with the MELAS causing disease mutation. hiPSC clones wild-type at 3243 position carried a GPM in mtDNA (m.9547 G>A in MitoA hiPSC clones or m.12005T>C in MitoB hiPSC clones). Mutant hiPSC clones at 3243 did not harbor those specific mutations, but others GPMs raised like m.4112T>C and m.5133A>G in MitoA hiPSC clones or m.1082A>G in MitoB hiPSC clones. We propose two possible explanations about the co-segregation of different mitochondrial genotypes in nuclear reprogramming. One is that the segregation of mtDNA is likely determined by nucleoid organization (mtDNA molecules and protein assemblages) during nuclear reprogramming (Gilkerson et al, 2008; Spelbrink, 2010). In this model, wild-type 3243 mtDNA molecules are associated with mutant 9547 mtDNA molecules in MitoA hiPSCs, likely through physical association in nucleoids. The second explanation is that both mutations m.3243A>G and m.9547G>A may not coexist in the same cell due to the detrimental effect of both mutations in mitochondrial function, mitochondria with both mutations are likely degraded through mitophagy (Narendra et al, 2008).”

The point of the paper is that the novel unknown mutations may be pathogenic and lead to functional defects, yet in the functional study of the iPSC-derived cardiomyocytes the authors focus a lot on the effect of the m.3424A>G mutation to the cellular respiration. The effect of the novel mutations could be emphasized more and less emphasis put on the known disease-causing pathogenic mutation, which was known to be present in the cells. It would be nice to see data from healthy donor cells.

We completely agree with reviewer’s comments about hiPSC-derived cardiomyocytes harboring MELAS causing disease mutation (A3243G), are the positive control in our experiments, that allowed us to properly analyzed any causal role in oxygen consumption in hiPSC-derived cardiomyocytes with GPMs revealed through nuclear reprogramming. We have addressed reviewer’s comment on page 11.
“Finally, we determined whether the mtDNA GPMs revealed through nuclear reprogramming, affected mitochondrial oxygen consumption rates in hiPSC clones and their differentiated counterparts. In the induced pluripotent state, all hiPSC clones exhibited similar basal and uncoupled respiration regardless their mtDNA genotype (Figure 4E), being in accordance with previous findings showing that hiPSCs are agnostic to disease-causing mtDNA mutations due to their low reliance in oxidative metabolism (Folmes et al, 2013). However, once the cells differentiate into cardiomyocytes, they rely mainly on OXPHOS to produce ATP. As expected, MitoA-61 and MitoA-69-derived cardiomyocytes containing m.3243A>G in MT-TL1 at 83% of mutation load, displayed basal and uncoupled respiration rates significantly lower than their wild-type counterparts (Figure 4F). Remarkably, MitoA-58-derived cardiomyocytes with GPM revealed during nuclear reprogramming (m.9547G>A in MT-CO3 at 70%) exhibited reduced basal and uncoupled mitochondrial oxygen consumption compared with their wild-type counterparts. The pathogenic effect of this GPM could lead to similar functional defects than MELAS causing disease mutation, when the mutation load reaches the 70%. MitoA-59-derived cardiomyocytes harboring the same mutation (m.9547G>A) at 40% heteroplasmy did not show reduced mitochondrial oxygen consumption, demonstrating the required mtDNA mutational threshold to show the mitochondrial defect (Figure 4F). It should be noted that not all the GPMs at high levels of mutation affected mitochondrial oxygen consumption in this study. The MitoA-217-derived cardiomyocytes containing the m.13918T>C mutation in MT-ND5 at 83% exhibited basal and uncoupled respiration rates similar to controls (Figure 4F). These results suggest that analysis of GPMs provides a useful tool for screening for potentially damaging mutations in mtDNA; however, functional analyses are necessary to confirm any causal role.”

Showing functional data from hiPSC clones derived from healthy donors would be a really interesting result. Based on the magnitude of variation in a wide spectrum of heteroplasmy across our large cohort of clones, it is expected that careful functional validation of these will indeed uncover and prioritize high-risk mutational burdens. We appreciate the reviewer’s suggestion and have a significant effort underway to comprehensively characterize these mutations based on the finding presented herein.

Minor points
The title says that mtDNA mutations are a source of intra-person human iPSC variability. This is a bit strong as it gives the idea that this is the source while the text it is stated as one potential source as it should be. While the mtDNA mutation are one of the sources, there definitely are other important sources as well, like mutations in the nuclear genome that have been shown to arise during reprogramming (several publications e.g Mayshar et al, Cell Stem Cell, 2010, Hussein et al, Nature 2011).

Following reviewer’s suggestion and following the limitation in number of characters restriction by EMBO journal (100 characters with spaces maximum), we have changed the title of our manuscript into:

“Natural underlying mtDNA heteroplasmy as a potential source of intra-person hiPSC variability”

Moreover, we have incorporated a new paragraph at the beginning of the discussion section, highlighting the idea of mtDNA mutations as a potential source of intra-patient variability, other sources can be responsible of this variability too, and both citations suggested by the reviewer have been incorporated in the final version of the manuscript.

“Functional variability among hiPSC clones derived from the same individual is one of the primary challenges to interpret and obtain reproducible results. Here, we reported mutations in mtDNA as a potential source in this intra-patient variability. It is important to note that other sources of variability have been reported previously in nuclear genes, like full or partial chromosomal aberrations (Mayshar et al, 2010) and copy number variants (Hussein et al, 2011).”

Referee #2:
This manuscript performed mtDNA NGS on 84 human iPSC lines from 19 donors. The analyses found that many of the lines harbored various levels of mutations, which are likely attributable to heterogeneity of the original fibroblasts. The authors also showed that some of these mutations resulted in decreased oxidative respiration in differentiated progenies. Based on these findings, the authors proposed a flow chart to evaluate iPSC clones prior to clinical applications. I found the work important and interesting. It should be published in EMBO journal provided that the authors properly address the following issues.

We appreciate reviewer’s recognition of the importance of our work and are thankful for the encouragement.

1. The authors argued that the different degree of mtDNA mutations was a source of intra-person variability of the iPSC lines. This may be true, but requires more careful discussion. Clonal variation of the iPSC lines includes differences in differentiation propensities and differences in functional performance after differentiation. As shown in figure 4, the authors did not observe the former. The authors did show that some mutations resulted in decreased oxidative respiration in cardiac myocytes, but whether this observation has any functional consequence remains unclear. Do these clones show any functional defects as cardiac myocytes? Without direct functional evidence, the title and the selection criteria shown in figure 5 would be too premature.

As reviewer #2 appropriately noted, the cardiac differentiation potential as reported by beating activity was not affected in hiPSCs harboring the mtDNA mutations included in this study. However, we showed a functional respiratory consequence of those mtDNA mutations in hiPSC-derived cardiomyocytes (low oxygen consumption rate compared with controls). Many mitochondrial disease patients develop variety of cardiomyopathies later in life due to developmental defects but likely ROS production and a defective aging process. This data motivates additional experimental work that is necessary to address these defects in vitro. We have included a new paragraph on page 15.

“The cardiac differentiation potential of the hiPSCs harboring those mutations, was not affected. It is well established that patients with mitochondrial disease develop cardiac manifestations like — hypertrophic and dilated cardiomyopathy, arrhythmias, left ventricular myocardial non-compaction, and heart failure (Meyers et al, 2013). More experimental work is necessary to modelling this defect in vitro.”

As reviewer #2 suggested, we have changed the title of our manuscript to accurately reflect the novel data presented herein:

“Natural underlying mtDNA heteroplasmy as a potential source of intra-person hiPSC variability”

Moreover, the final version of our manuscript does not contain the Figure 5, we agree with the reviewer’s comment about the premature nature of that figure in the context of this work and we regret to have included it in the previous version of the manuscript. Ongoing characterization of a large number of mutant clones will be required to justify this triage model.

2. The authors should compare their results with those by Mitalipov and colleagues published earlier this year. Most notably, the two works differ sharply in age dependency of the mtDNA mutations. The authors provided nice figures in their responses to the reviewers in their previous submission, which I believe should be included in the manuscript.

Following reviewer’s recommendation to better highlight the novelty of this work, we have compared our results from recently Mitalipov’s paper, pointing out the main difference in the accumulation of mtDNA mutations in hiPSCs is not age dependent, on page 14 of the final version of our manuscript. We are able to show data from individuals as young as one month of age at the time of tissue collection. We have included the figure representing the % of mutation load versus the donors’ age at skin biopsy in the Expanded View Figure 2, as the reviewer suggested. This is extremely important to have highlighted in this manuscript. Thank you.

“Our results show that low levels of heteroplasmic mtDNA variants are revealed in nuclear reprogramming regardless of donor’s age, as we found GPMs exhibiting heteroplasmy levels in
excess of 10% in hiPSC clones-derived from individuals from one month of age to 44 years of age in control group, supporting the Universal Heteroplasmy Theory as the process responsible for the differences exposed in mtDNA during nuclear reprogramming, rather than an accumulation of somatic mutations with age (Kang et al, 2016). Our findings demonstrate that hidden mtDNA heteroplasmy can profoundly affect hiPSC function when differentiated into cardiac lineages and is consistent with emerging evidence that indicates a generalizable mechanism inherent to clonal expansion of hiPSC clones (Kang et al, 2016). Additionally, our data establishes that all ages of patients and independent of mitochondrial disease phenotype are vulnerable to mtDNA load. Conversely, low heteroplasmy hiPSC clones are commonly identified even from mitochondrial patients expected to have high heteroplasmy based on clinical presentation.”

Accepted 24 June 2016

Thank you for submitting the revised version of your manuscript to us. I appreciate the introduced changes, and I am happy to accept your manuscript for publication in The EMBO Journal
### 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 authorship guidelines on Data Presentation.

2. **Captions**
   - Each figure caption should contain the following information, for each panel where they are relevant:
     - A description of the experimental system investigated (e.g., 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, tissues, 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 or unpaired), simple j2 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 value = x but not P value < x; definition of “center values” as median or average; definition of error bars as S.D. or S.E.M.

   Any descriptions relating to 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.

### B. Statistics and general methods

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<tr>
<th>Question</th>
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<td>1. a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?</td>
<td>Yes</td>
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<td>1. b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.</td>
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<td>2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?</td>
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<td>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.</td>
<td>Yes</td>
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<td>3. For animal studies, include a statement about randomization even if no randomization was used.</td>
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<tr>
<td>4. a. Were any steps taken to minimize the effects of subjective bias during group allocation (e.g., blinding of the investigator)? If yes please describe.</td>
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<td>4. b. For animal studies, include a statement about blinding even if no blinding was done.</td>
<td>Yes</td>
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<td>5. For every figure, are statistical tests justified as appropriate?</td>
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<td>6. a. Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.</td>
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<td>6. b. If an estimate of variation within each group of data?</td>
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<td>7. Is the variance similar between the groups that are being statistically compared?</td>
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To show that antibodies were profiled for use in the system under study, ensure spectra and species, provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile, e.g., Biolegend (see link at top right). Diagrammed (see link list at top right).

Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. All QC clones were tested by progeny analysis with the corresponding parental lines. All lines are negative for mycoplasma contamination.

D- Animal Models

- Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.

- For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.

- We recommend consulting the ARRIVE guidelines (see link list at top right) (PloS Biol. 8, e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. Please confirm compliance.

E- Human Subjects

- Identify the committee(s) approving the study protocol.

- 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.

- For publication of patient photos, include a statement confirming that consent to publish was obtained.

- Report any restrictions on the availability (public or on the web) of human data or samples.

- Report the clinical trial registration number (at ClinicalTrials.gov or equivalently), where applicable.

- 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.

- 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.

F- Data Accessibility

- Provide accession codes for deposited data. See author guidelines, under ‘Data Deposition’.

  Data deposition in a public repository is mandatory for:
  a. Proteins, DNA and RNA sequences
  b. Macromolecular structures
  c. Crystallographic data for small molecules
  d. Functional genomics data
  e. Proteomics and molecular interactions

- Deposition is strongly recommended for all 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).

- Access to human clinical and genetic datasets should be provided with all the infrastructure 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 biomedical repositories such as: EMDB, EBI (EMBL-EBI) and NHGRI-EBI dbGaP; the relevant accession numbers should be included in the paper. The relevant accession numbers or links should be provided. Where possible, standardized data formats (BIDMC, iCARE) should be used instead of scripts (e.g., MATLAB). Authors are strongly encouraged to follow the MIABML 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 DB-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.

- 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


  Referenced Data


  AP-MS analysis of human histone deacetylase interactions in COS-7 cells (2013). PFRE P1000028

  A multiprotein model that are central and integral to a study should be tested without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. Where possible, standardized format (BIDMC, iCARE) should be used instead of scripts (e.g., MATLAB). Authors are strongly encouraged to follow the MIABML 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 DB-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.

G- Dual use research of concern

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