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TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury

Fargol Mazaheri, Nicolas Snaidero, Gernot Kleinberger, Charlotte Madore, Anna Daria, Georg Werner, Susanne Krasemann, Anja Capell, Dietrich Tr¸mbach, Wolfgang Wurst, Bettina Brunner, Sebastian Bultmann, Sabina Tahirovic, Martin Kerschensteiner, Thomas Misgeld, Oleg Butovsky, and Christian Haass

Corresponding author: Christian Haass, Ludwig-Maximilians-University DZNE

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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 08 February 2017

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. We have now received the full set of referee comments and cross-comments that is pasted below.

As you will see, the referees acknowledge that the findings are interesting. However, they also point out short-comings and that the gene expression changes in TREM2 KO microglia should be more carefully analyzed and discussed. A full list with the expression changes of all tested genes should be provided too. Regarding the functional assay suggested by referee 2, referee 3 does not agree that this is crucial, and while certainly welcome, this is therefore not a strict requirement for publication of the study here.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

REFEREE REPORTS

Referee #1:

The manuscript proposes that in the absence of Trem2 microglia better maintains homeostasis, primarily with respect to chemotxis.
The authors use several functional assay that are nice, but no mechanistic suggestion was provided between regulation of homeostatic genes and impaired chemotaxis.

Basically the authors used NanoString RNA expression analysis and several functional analyses showing impaired chemotaxis and migration toward chemo-attractants and apoptotic neurons.

In general, the gene expression changes in Trem2 KO relative to WT seems very moderate with upregulation of around ~1.2 fold of microglia hallmark genes (Fig 1D) with relatively high variation between replicates (based on the heatmap views in Figure 1A and 1E).

More than quarter of the genes tested were found to be up or down regulated in Trem2 KO which seems quite surprising. A scatter plot showing the average expression of 482 genes in WT and Trem2 KO would help evaluating the overall changes and similarities in gene expression. The "disturbed expression" profile should be further characterized, which genes were down-regulated?

The functional analysis shows impairs chemotaxis in Trem2 KO mice, whether this lack of function is due to a 'lock' in a resting state remains to be shown. Trem2 signalling cue or downstream molecular effect can strengthen this work.

Minor points:
1. Figure 4 is missing annotation of the channels in panels A and B and y-axis label in panel C.

Referee #2:

Mozaheri set out to understand how TREM2 mutations affect Alzheimer's disease (AD). Because these mutations seem to result i loss of function in microglia, in which the gene is predominantly expressed, they carry out a gene expression analysis in TREM2 deficient microglia. They report that these mutant microglia show a resting state based on expression of a number of microglial "signature genes" and hypothesize that TREM2 deficient microglia are unable to become activated in response to stress or injury. They find Trem2 deficiency impairs microglial chemotaxis towards amyloid plaques in situ, towards chemotactic factors in vitro, or towards apoptotic neurons in vivo. Lastly, they show that microglia lacking Trem2 fail to grow processes in response to a focal laser injury.

Overall, I think the findings are convincing and solid and add to a better understanding of Trem2 function. The strength is in the various functional assays but the study does not tie back to the Trem2 mutations linked to AD.

It would be important to show at least some of the functional assays with cells expressing mutant Trem2. Ideally, this would be achieved by using microglia from Trem2mutant knockin mice, if available, or, more likely, from Trem2 ko microglia reconstituted with wt or mutant Trem2.

Referee #3:

Referees are asked to supply answers to the following questions, with brief accompanying comments where appropriate:

1. Does this manuscript report a single key finding? YES/NO
If YES, please describe it in one sentence.

Yes. This manuscript describes how Trem2 deficient microglia are affected in their migratory capacity

2. Is the reported work of significance (YES), or does it describe a confirmatory finding or one that

has already been documented using other methods or in other organisms etc (NO)? YES

3. Is it of general interest to the molecular biology community? YES

If YES, please say why, in a single sentence. If NO, please state which more specialized community you feel it is aimed at (or none), in a single word or phrase.

Trem2 is clearly implicated in neuropathalogical disorders and considered a therapeutic target in AD, therefore characterization of its biological functions is relevant

4. Is the single major finding robustly documented using independent lines of experimental evidence (YES), or is it really just a preliminary report requiring significant further data to become convincing, and thus more suited to a longer¨format
article (NO)? YES

The manuscript by Mazaheri et al. describes how Trem2 deletion affects the homeostatic-microglia specific mRNA signature. Furthermore a role for the Trem2 protein in chemotaxis is described. Overall in the manuscript the effects of Trem2 deletion on cell motility (both chemotaxis of whole cells, as well as outgrowth of processes) have been convincingly documented. The authors have used several techniques to determine chemotaxis as well as different methods of deletion & rescue of Trem2. Specific inhibitors targeting downstream signaling molecules further strengthen the claim that Trem2 is important for efficient chemotaxis in microglia.

However, the data describing transcriptional changes in response to Trem2 deletion are less convincing or less well descibed, especially the link between the alterations in transcription and the observed motility phenotype is not particularly clear.

Major:

1. Title: The short title much better describes the contents of the manuscript, the long title has two issues:
- Responses to neuronal injury: In the experiment where apoptotic neurons were injected, only migration of microglia has been assessed.
- 'enhances the homeostatic mRNA signature' What does that specifically mean? The data in figure 1 is presented in such a way that significant changes between resting wt and Trem2 deficient microglia are highlighted. There is no data presented to suggest that homeostatic functions are enhanced in Trem2 deficient microglia, rather they are altered/negatively affected.
2. The significant expression changes presented in figure 1 have a very low fold change. Preferably their significance should be validated using a separate approach (eg. qPCR for some of the relevant genes).
3. As it is, are the differences in fig1D biologically relevant? These data should be discussed more extensively. Are the relatively small differences in transcription the result of the presence of a small proportion of microglia in the wt that are activated (and therefore in the wt the average signal in homeostatic genes is lower), while the cells in the Trem2 KO are more homogeneous in their expression profile? Or is the idea that all cells express the genes slightly higher?
4. In figure 1E, why was an FDR of 0.158 used as a cutoff? What does the data look like when a more conventional FDR<0.05 is applied?
5. The effect of the altered genes listed in table 1 could be explored more and described. Within a specific GO-category similar numbers of genes are up- and down-regulated. How does that fit the hypothesis?
6. The methods should be described better. For example: how were the injected apoptotic neurons generated? How many cells were injected? Does the injection volume damage the resident neurons? The saline injected controls should be depicted in the results.
Another example: the culture of N9 cells is not described in the methods, neither is the generation of the N9 KO and Trem2-rescue.
7. Figure 3 would benefit from a figure panel showing the expression levels (preferably protein) of Trem2 in each tested condition

Minor:

1. A full list of all 400 tested genes (Fig 1A) and the effect on their transcription should be provided. Also a list containing all genes represented in Fig 1E should be provided.
2. Typos/double spaces

Cross-comments from referee 2:

The comments from reviewer 3 seem reasonable and can mostly be addressed in writing. Point 2 is the only one that requires an experiment and seems reasonable.

Cross-comments from referee 3:

I understand the point raised, but the amount of additional work would be excessive for the scientific advancement it would most likely provide. Rescue experiments of primary microglia by transgene expression is very hard and not common. Microglia can be transcend lentiviral but it leads to strong activation, changing their phenotype. Difficult and somewhat messy experiments.

My main issue is with figure 1, that is not very convincing, and the data are not very wel presented. In terms of quality of the paper, I think that addressing fig1 will improve the paper much more than Trem2 mutant rescue experiments in Trem2 KO primary microglia.

1st Revision - authors' response 03 March 2017

Many thanks for inviting us to submit a revised version of our manuscript originally entitled "TREM2 deficiency enhances the homeostatic mRNA signature of microglia and impairs chemotaxis and responses to neuronal injury". We fully agree with the reviewers that the mRNA expression data in Fig. 1 were not sufficiently described and in part even over-interpreted. We have therefore restructured the entire manuscript and focus now on microglial deficits in terms of chemotaxis and response to neuronal injury. In addition we toned down the "stabilization" of the homeostatic signature and only conclude that the TREM2 knockout does not suppress the homeostatic mRNA signature, and that failure to do so may be one reason why TREM2 knockout microglia is unable to mount appropriate responses to neuronal injury and chemotactic stimuli. Consequently, and in line with the suggestion of reviewer #3 we changed the title of the manuscript accordingly to: "TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury." Point-by-point response to referees' concerns (responses in italics):

Referee #1

The authors use several functional assay that are nice, but no mechanistic suggestion was provided between regulation of homeostatic genes and impaired chemotaxis.

We discuss this point on the example of Sall1 and TGFb1 signaling in the Discussion on page 9. In addition we have restructured the entire manuscript and toned down and corrected the interpretation of the data on the homeostatic mRNA signature also in accordance with the editor and reviewer #3. We now focus on the functional consequences of dysregulation of the genes in the "chemotactic gene cluster".

In general, the gene expression changes in Trem2 KO relative to WT seems very moderate with upregulation of around ~1.2 fold of microglia hallmark genes (Fig 1D) with relatively high variation between.

We now clarify this point by only stating that the homeostatic signature is not suppressed by Trem2 knockout, and that this may be one of the reasons why microglia fail to respond to chemotactic stimuli and neuronal injury. We adjusted the first paragraph of the Results section accordingly, and added a new paragraph at the end of the Results, which now carefully describes the stable homeostatic mRNA signature in Trem2 knockout microglia. Moreover, we adjusted the title

accordingly to accurately display the main conclusion of our manuscript (in agreement with referee #3).

More than a quarter of the genes tested were found to be up or down regulated in Trem2 KO which seems quite surprising. A scatter plot showing the average expression of 482 genes in WT and Trem2 KO.

The scatter plot is now provided in the new Fig. 1A. Although we agree that the overall changes observed are relatively minor, application of pathway enrichment analysis separately to significantly up- and downregulated genes from the NanoString-based screen revealed that in particular downregulated genes contribute to chemotaxis and migration (compare the top significant functional categories of the new TabEV2 and TabEV3). Moreover, functional analyses of chemotaxis at multiple levels fully confirmed our prediction from the mRNA analysis. This is now described in detail within the first paragraph of the Results section.

The functional analysis shows impairs chemotaxis in Trem2 KO mice, whether this lack of function is due to a 'lock' in a resting state remains to be shown.

We agree with the reviewer. We have therefore restructured the entire manuscript and focus now on microglial deficits in terms of chemotaxis and neuronal response. In addition we toned down the "stabilization" of the homeostatic signature and only conclude that the TREM2 knockout does not suppress the homeostatic mRNA signature, and that failure to do so may be one reason why TREM2 knockout microglia is unable to mount appropriate responses to neuronal injury and chemotactic stimuli. Consequently, and in line with the suggestion of reviewer #3 we changed the title of the manuscript accordingly: "TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury".

Minor points:

1. Figure 4 is missing annotation of the channels in panels A and B and y-axis label in panel C.

We added the annotation of the channels in Fig. 4A and B and added the missing description of the y-axis in C.

Referee #2

Overall, I think the findings are convincing and solid and add to a better understanding of Trem2 function. The strength is in the various functional assays but the study does not tie back to the Trem2 mutations linked to AD. It would be important to show at least some of the functional assays with cells expressing mutant Trem2. Ideally, this would be achieved by using microglia from Trem2 mutant knockin mice, if available, or, more likely, from Trem2 ko microglia reconstituted with wt or mutant Trem2.

In our experience it is exceedingly difficult/impossible to reconstitute primary microglia. This is also in line with referee's #3 opinion to this point. Nevertheless, we want to refer to our manuscript entitled: "The FTD-like syndrome causing TREM2 T66M mutation impairs microglia function, brain perfusion and glucose metabolism," which is currently under consideration at EMBO J. Here we introduced the TREM2 p.T66M mutation with the CRISPR/Cas9 technology into the genome of a mouse. Microglia of this mouse model indeed show profound phenotypic loss-of-function changes and some of them such as the failure to form microglial nodules during ageing may be consistent with reduced chemotactic migration.

Referee #3

However, the data describing transcriptional changes in response to Trem2 deletion are less convincing or less well described, especially the link between the alterations in transcription and the observed motility phenotype is not particularly clear.

We agree with the reviewer (and referee 1, who raised very similar concerns). We have therefore restructured the entire manuscript and focus now on microglial deficits in terms of chemotaxis and

neuronal response. In addition we toned down the "stabilization" of the homeostatic signature and only conclude that the TREM2 knockout does not suppress the homeostatic mRNA signature, and that failure to do so may be one reason why TREM2 knockout microglia is unable to mount appropriate responses to neuronal injury and chemotactic stimuli. Consequently, and in line with the suggestion of reviewer #3 raised below we changed the title of the manuscript accordingly: "TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury".

Major points:

1. Title: The short title much better describes the contents of the manuscript.

We agree with the referee and have changed the title accordingly.

2. The significant expression changes presented in figure 1 have a very low fold change. Preferably their significance should be validated using a separate approach (eg. qPCR for some of the relevant genes).

We clarify this point by only stating that the homeostatic signature is not suppressed by Trem2 knockout (what is clearly proven by quantitative RNA analysis), and that this may be one of the reasons why microglia fail to respond to chemotactic stimuli and neuronal injury. We adjusted the first paragraph of the Results section accordingly, and added a new paragraph at the end of the Results, which now carefully describes the stable homeostatic mRNA signature in Trem2 knockout microglia.

3. As it is, are the differences in fig1D biologically relevant? These data should be discussed more extensively.

See our answer to point 2 of this referee.

4. In figure 1E, why was an FDR of 0.158 used as a cutoff? What does the data look like when a more conventional FDR<0.05 is applied?

The requested data are now presented in Expended View Fig. 1.

5. The effect of the altered genes listed in table 1 could be explored more and described. Within a specific GO-category similar numbers of genes are up- and down-regulated. How does that fit the hypothesis?

We have now intensified the discussion of the dysregulated genes (see new paragraph one in the Results section) and added three new tables (EV Table 1 to 3) showing all up- and down regulated genes. Compensatory mechanisms may [be] responsible for the similar amounts of genes up- and down regulated.

6. The methods should be described better. For example: how were the injected apoptotic neurons generated? How many cells were injected? Does the injection volume damage the resident neurons? The saline injected controls should be depicted in the results.

These methods are now described in detail as requested.

Another example: the culture of N9 cells is not described in the methods, neither is the generation of the N9 KO and Trem2-rescue.

This is now adjusted accordingly and we also refer to the publication of the N9 mutant cell line in our previous paper (Xiang et al., EMBO Mol Med, 2016).

7. Figure 3 would benefit from a figure panel showing the expression levels (preferably protein) of Trem2 in each tested condition.

We have added the requested western blot to Fig. 3 (see new Fig. 3E).

Minor: A full list of all 400 tested genes (Fig 1A) and the effect on their transcription should be provided. Also a list containing all genes represented in Fig 1E should be provided.

This has been provided by the addition of the new EV Table 1.

Taken together we believe that we have carefully addressed all points raised by the reviewers. Again, thank you very much for considering our manuscript.

2nd Editorial Decision 23 March 2017

Thank you for the submission of your revised manuscript. We have now received the enclosed report from referee 3 who was asked to assess it and who supports the publicaiton of your revised study. I only would like you to incorporate a few minor changes before we can proceed with its official acceptance. We will publish your manuscript as a full article, given the 6 main figures and the separate results and discussion sections.

Please relabel the 3 EV tables "Dataset 1, 2, 3" and include the table legend in the first cell of the excel file. Please make sure that all 3 Datasets and Table 1 are cited in the manuscript text.

Please add a title to Table 1.

Figures 3 and 6 do not specifc "n", please add this information.

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

REFEREE REPORTS

Referee #3: Mazaheri and coworkers addressed the points raised in the first review report adequately and sufficiently. One remark, after reanalyzing the data with an FDR of 0.05, several genes labelled as important in Fig 1b now are no longer significantly different between WT and TREM2 KOs. Sall1 and Tgfbr1, genes discussed fairly extensively in the manuscript, however are still significantly different.

2nd Revision - authors' response 27 March 2017

The authors made the requested changes and submitted the final version of their manuscript.

3rd Editorial Decision 31 March 2017

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.

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Corresponding Author Name: Christian Haass	
Journal Submitted to: EMBO Reports	
Manuscript Number:	

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 χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - 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

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

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 chosen according to our experience with in vitro assays, in vivo and organotypic slice cultures and cell biological and biochemical experiments.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	for the in vivo experiments, we used the minimal numbe of mice that was sufficient for determining significant differences per group; for in vivo live imaging experiments we increased the N number by introducing a few laser lesions per mouse brain in areas far from each other, see figure legends where sample size and corresponding statistics are detailed
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	in vivo: one Trem2 knock out mouse had very low GFP expression and the signal was not enough for reliable quantifications. Therefore, this mouse was excluded, we also excluded one WT that was measured on the same day, because its knockout counterpart was not analyzed.
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.	The animal surgeon was blinded during the surgery. Several scientists looked at data blindly and reached the same conclusion.
For animal studies, include a statement about randomization even if no randomization was used.	mice were chosen randomly from a pool of genotyped mice at the same age.
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.	yes, for example in vivo and ex vivo experiments were shown to several colleagues blindly, and they could identify the migration impairment in knockout without knowing its genotype
4.b. For animal studies, include a statement about blinding even if no blinding was done	please see 4.a.
5. For every figure, are statistical tests justified as appropriate?	Yes. All statistical tests are mentioned in the figure legends and also described in the experimental procedures.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	yes. Statistical tests done for each experiment are mentioned in figure legends.
Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

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).	Antibodies for microglia isolation and stainings were provided by the group of Dr Oleg Butovsky. Antibody for Trem2 western blot was generated in the Haass laboratory and already published in Kiang et. al. 2016, EMBO Mol. Med.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N9 cells were available at Haass laboratory, and frequently tested for Mycoplasma (and in all cases they were negative for Mycoplasma)

* 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.	For RNA profiling by Nanostring we used male mice at the age of 3.5-4 months old. For in vivo migration 3-4 months old (mixed gender: due to unavailability we did not use only male). For ex vivo: p5 mice as published in Daria et. al. 2016 EMBO Journal.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal experiments were performed in accordance to local animal handling laws. Housing conditions included standard pellet food and water provided ad libitum, 12---hour light---dark cycle at temperature of 22 C with cage replacement once per week and regular health monitoring. APP\PS1 mice (Radde R et al. 2006) were generated by Jucker's group. Trem2 knockout mice (Turnbull IR et al. 2006) were provide by Colonna's group.
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.	

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	No experiments with human subjects were conducted.
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.	No experiments with human subjects were conducted.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	No experiments with human subjects were conducted.

14. Report any restrictions on the availability (and/or on the use) of human data or samples.	No experiments with human subjects were conducted.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	No experiments with human subjects were conducted.
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.	No experiments with human subjects were conducted.
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.	No experiments with human subjects were conducted.

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