THE EMBO JOURNAL

BGL3 lncRNA mediates retention of the BRCA1/BARD1 complex at DNA damage sites

Huadong Pei, Zhaohua Hu, Shaojie Mi, Ting Zhao, Changmin Peng, Yihan Peng, Lulu Chen, Wenge Zhu, Yi Yao, Xiangpan Li, Qibin Song, Chenxi Jia and Xinzhi Li

Review timeline:

Submission date:	27th November 2019
Editorial Decision:	20th December 2019
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Editorial Decision:	24th March 2020
Revision received:	26th March 2020
Accepted:	27th March 2020

Editor: Hartmut Vodermaier

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 20th December 2019

Thank you for submitting your manuscript on BGL3 lncRNA in homologous recombination repair for our editorial consideration. It has now been evaluated by three expert referees, who all appreciate the overall technical quality of the work as well as the potential interest of your findings. Pending satisfactory revision of a number of specific issues, we shall therefore be happy to consider this study further for EMBO Journal publication. In particular, points 2, 5, 7 & 8 of referee 1, and points 1 & 2 of referee 3 will require in-depth addressing. Furthermore, the referees note that the manuscript needs careful proofreading/editing

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REFEREE REPORTS
Referee #1:

In this manuscript, the authors present evidence that BGL3 lncRNA plays an important role in BRCA1-BARD1 mediated DNA repair. This is a new and interesting topic as more evidence about lncRNA in DNA repair begins to emerge. For example, p53-responsive lncRNA GUARDIN stabilizes BRCA1, facilitates BRCA1-BARD1 complex formation and functions in maintaining genomic integrity. In this study, the authors uncover another lncRNA-BGL3, which also regulates genome stability. Briefly, the authors provide evidence that 1) BGL3 interact with BARD1 and PARP1 directly; 2) BGL3 is required for homologous recombination (HR) and genome stability; 3) the recruitment of BRCA1, BARD1, CtIP, RPA, RAD51 upon UV laser micro-irradiation is reduced by knocking down BGL3; 4) BGL3 promotes complex formation between BARD1-HP1y and BARD1-RAD51, and 5) The early recruitment of BGL3 is dependent on PARP1 and its DNA binding domain, but HP1y plays a role in the retention of BGL3 at late times.

Based on the above, the authors propose a model in which BGL3 is recruited by PARP1 and functions as a scaffold to promote the interaction of BARD1 with HP1 and RAD51 relevant for DNA end resection and the execution of HR.

- Specific major concerns essential to be addressed to support the conclusions

The conclusions are well supported by the data, and in general, the appropriate controls were performed. Specific suggestions are listed below, but the manuscript requires thorough editing.

Specific Comments
1. There are several typos and grammatical errors in the manuscript:
a. The word "respectively" is used incorrectly several times, including in the abstract and intro.
b. The word "an" is omitted before the phrase "early timepoint" throughout the text.
c. CtIP is sometimes written as CTIP.
d. There is frequently no space between the last word in a sentence and the in-text citation. For example, at the bottom of pg 5, "repair processes(Zhang & Peng, 2015)" should be "repair processes (Zhang & Peng, 2015)."
e. The RESULTS subheading is spelled incorrectly.
f. DDR is first used on pg 5 but not defined until pg 7.
g. The end of the major paragraph on pg 7 is poorly worded. It should be "DNA metabolism and cell cycle regulation," and in what way do the authors mean cytoskeleton? The authors claim these are the "main" functions found through GO-term analysis, but based on Fig. EV1D, BGL3 was relatively evenly distributed through the listed GO-terms, with an enrichment in DNA damage. Please clarify this sentence.
h. PARP1 is spelled wrong on pg 15.
2. Provide evidence that the lncRNA being pulled down by the RNA antisense pulldown experiment is indeed BGL3.
3. What is Fig. EV1E adding to what is already presented in Fig. 1A? Consider removing Fig. EV1E.
4. How do the authors explain the inconsistencies in the BARD1 truncations and their interactions with BGL3? For example (in Fig. EV2B), BGL3 was enriched in the 566-777 fragment, but longer BARD1 constructs had ~2-fold less BGL3 enrichment. Similarly, and contradictory, the BARD1 1-555 construct was dramatically more enriched in the Fig. 1F.
5. A non-denaturing pulldown of BGL3 would help show the proteins that are interacting with BGL3 and functioning in their appropriate complexes. For example, BRCA1 should be pulled down with BGL3 through BARD1.
6. In Fig 1C and D, BRCA1 species in 10-Gy IR treated samples are the same as the ones in untreated samples. One would expect BRCA1 to be post-translationally modified after DNA damage.
7. Knockdown of BARD1 should have a dramatic effect on HR in the DR-GFP reporter assay. However, the authors saw only 50% reduction (Figure 4D). The same concern applies to CtIP depletion (Figure 3A).
8. A positive control for PARP-inhibitor sensitivity in the Fig. EV3C cell survival assays would support the results.

- Minor concern

- In the section of introduction, many references are not the correct ones to cite, such as those of PARP inhibitors and RING heterodimer.

Referee #2:

This is an interesting paper describing the role of BGL3 lncRNA in regulation of homologous recombination. The authors found that BGL3 binds to DBD domain of PARP1, and the C-terminal BRCT domain and an internal region of BARD1. It seems that the PARP1 is required for BGL3 recruitment to DSBs and that BGL3 is required for retention of BARD1-BRCA1 at DSBs. BGL3 is required for HR, especially resection step of HR, and cellular resistance to DNA damaging agents and PARP inhibitors. These are novel and interesting findings.
Generally, the experiments were well done, and the results are convincing.

Major points:
1) The authors should discussion about clinical relevance of BGL3 lncRNA.
Are there any BGL3 mutations found in human cancers?
How about chemosensitivity (or prognosis) of tumors with BGL3 mutations?

Minor points:
1) Typo : P.12 L.14 which is import for --> which is important for
2) P.13 .L. 12 we will determine --> we determined

Referee #3:

The manuscript by Hu et. al. describes the role of a lncRNA, BGL3, in promoting cell survival upon DNA damage. Mechanistically, the authors show that BGL3 binds to and recruits kay players in the DNA damage response including BRCA1 and BARD and that its recruitment to DNA damage sites is dependent on PARP activity. Functionally, the authors show that BGL3 depletion leads to enhanced sensitivity to DNA damage and reduced recruitment of DNA damage response proteins to the damage sites.
The manuscript is well written and a pleasure to read. The data is clean and convincing in particular, the mechanistic assays related to the lncRNA-protein interaction are well performed and described. The findings are novel and important to the lncRNA and DNA damage field. I am enthusiastic about this work but do have some major concerns (require experiments):
1. The function of BGL3 in protecting cells from DNA damage is a major finding and should be better supported by using at least two cell lines per assay. In addition, it is not clear if BGL3 deficient cells are dying from DNA damage or just arrest. The manuscript will benefit from more thorough description of the consequence of BGL3 depletion and DNA damage to cell survival.
2. The authors use siRNA to manipulate BGL3 and, for example, follow the recruitment of various proteins to damage sites within the nucleus. siRNA function by depleting the cytosolic RNA pool leading to the question of where is the damage site localized BGL3 RNA coming from? This question is made more relevant by the finding that BGL3 expression is not induced by damage and therefore, most likely, recruited BGL3 is 'old RNA'. One way to address this question is fractionate the cell into cytosolic, nuclear and chromatin fractions and probe for BGL3 before and following DNA damage.

1st Revision - authors' response — 9th February 2020

We thank the referees for their positive comments and insightful suggestions. We have addressed all their concerns in our revised manuscript, and respond to each point below.

Referee #1:

In this manuscript, the authors present evidence that BGL3 lncRNA plays an important role in BRCA1-BARD1 mediated DNA repair. This is a new and interesting topic as more evidence about lncRNA in DNA repair begins to emerge. For example, p53-responsive lncRNA GUARDIN stabilizes BRCA1, facilitates BRCA1-BARD1 complex formation and functions in maintaining genomic integrity. In this study, the authors uncover another lncRNA-BGL3, which also regulates genome stability. Briefly, the authors provide evidence that 1) BGL3 interact with BARD1 and PARP1 directly; 2) BGL3 is required for homologous recombination (HR) and genome stability; 3) the recruitment of BRCA1, BARD1, CtIP, RPA, RAD51 upon UV laser micro-irradiation is reduced by knocking down BGL3; 4) BGL3 promotes complex formation between BARD1-HP1y and BARD1-RAD51, and 5) The early recruitment of BGL3 is dependent on PARP1 and its DNA binding

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h. PARP1 is spelled wrong on pg 15.
Response： We have proofread the manuscript and corrected all the typos and grammatical errors. We have rewritten the end of the major paragraph on pg. 7.

2. Provide evidence that the lncRNA being pulled down by the RNA antisense pulldown experiment is indeed BGL3.
Response: We sequenced the RT-PCR product (Fig EV1B) to confirm the results, and the lncRNA being pulled down by the RNA antisense pulldown experiment is indeed BGL3 (Appendix material 1). We also did Q-RT-PCR to analyze the lncRNA being pulled down in the “BGL3 antisense group” (BGL3 group) and the control group (RMRP group). Again, our results clearly indicated that BGL3 is pulled down in the “BGL3 antisense group”, but not in the control group (Fig EV1C).

3. What is Fig. EV1E adding to what is already presented in Fig. 1A? Consider removing Fig. EV1E.
Response: We removed Fig. EV1E in the revised version.

4. How do the authors explain the inconsistencies in the BARD1 truncations and their interactions with BGL3? For example (in Fig. EV2B), BGL3 was enriched in the 566-777 fragment, but longer BARD1 constructs had ~2-fold less BGL3 enrichment. Similarly, and contradictory, the BARD1 1-555 construct was dramatically more enriched in the Fig. 1F.
Response: We redid these experiments and now show more representative figures. As shown in Fig 1F and Fig EV2C, the BARD1 566-777 construct was more enriched. The truncated BARD1 BRCT domain (amino acids 566-777) structure may be a little different from the full-length BARD1 structure, which binds more BGL3. But we still conclude that BARD1 bound to BGL3 through its C-terminal BRCT domain (amino acids 566-777) and an internal region (amino acids 127-424).

5. A non-denaturing pulldown of BGL3 would help show the proteins that are interacting with BGL3 and functioning in their appropriate complexes. For example, BRCA1 should be pulled down with BGL3 through BARD1.
Response：This is really a helpful suggestion. A non-denaturing pulldown assay clearly showed that BRCA1was pulled down with BGL3 through BARD1 (Fig EV2B).

6. In Fig 1C and D, BRCA1 species in 10-Gy IR treated samples are the same as the ones in untreated samples. One would expect BRCA1 to be post-translationally modified after DNA damage.
Response：BRCA1 was indeed modified after DNA damage. But because of the limited resolution of the SDS-PAGE gel, sometimes the BRCA1 band shift was not visible (Lee et al, 2000; Zhang et al, 2016). As shown in Fig EV2B, BRCA1 post-translational modification (such as S1524 phosphorylation) is still there, and this modification was induced by DNA damage.

7. Knockdown of BARD1 should have a dramatic effect on HR in the DR-GFP reporter assay. However, the authors saw only 50% reduction (Figure 4D). The same concern applies to CtIP depletion (Figure 3A).
Response： We repeated the HR assay, and now provide more representative results. As shown in Figure 3A and 4D, knockdown of CtIP/ BARD1 resulted in a marked decrease in HR (more than 50% reduction).

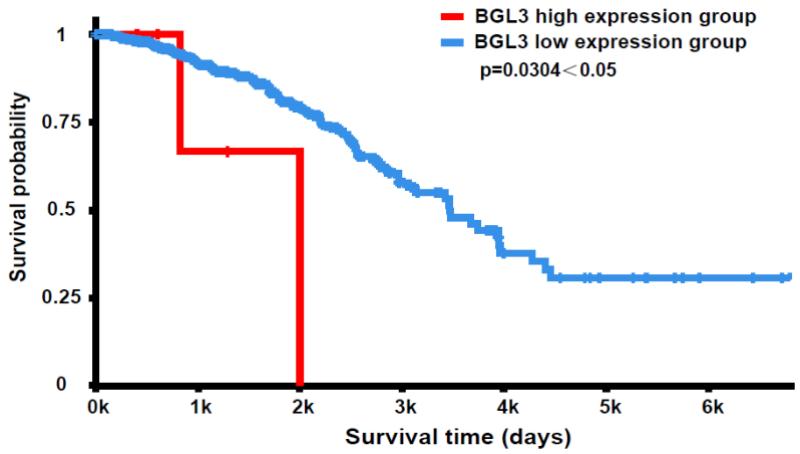
8. A positive control for PARP-inhibitor sensitivity in the Fig. EV3C cell survival assays would support the results.
Response：Thanks for the comment and helpful suggestion. We included BARD1 knockdown as a positive control for PARP-inhibitor sensitivity in the survival assays shown in Fig. EV4A.

- Minor concern
- In the section of introduction, many references are not the correct ones to cite, such as those of PARP inhibitors and RING heterodimer.

Response：We have now checked all the citations carefully and corrected all the wrong references.

Referee #2:
This is an interesting paper describing the role of BGL3 lncRNA in regulation of homologous recombination. The authors found that BGL3 binds to DBD domain of PARP1, and the C-terminal BRCT domain and an internal region of BARD1. It seems that the PARP1 is required for BGL3 recruitment to DSBs and that BGL3 is required for retention of BARD1-BRCA1 at DSBs. BGL3 is required for HR, especially resection step of HR, and cellular resistance to DNA damaging agents and PARP inhibitors. These are novel and interesting findings. Generally, the experiments were well done, and the results are convincing.

Major points:
1) The authors should discussion about clinical relevance of BGL3 lncRNA. Are there any BGL3 mutations found in human cancers?
How about chemosensitivity (or prognosis) of tumors with BGL3 mutations?
Response：We thank the review for these comments and suggestions. We have added some discussion of this point in our revised manuscript. We could not find significant BGL3 mutations in the LncRNA-related database (http://bioinformatics.mdanderson.org/main/TANRIC:Overview) (Li et al, 2015). But BGL3 levels were negatively correlated with overall survival among patients with breast cancer (see figure below). Perhaps overexpression of BGL3 enhances DNA repair capability, and thus promotes tumor progression and drug resistance, but this question needs further study. This is similar to what is known about RAD51. RAD51 is essential for HR repair, but it is overexpressed in several cancer types, such as breast and pancreatic cancers and associated with enhanced tumor progression and drug resistance (Henning & Sturzbecher, 2003; Klein, 2008).



Minor points:
1) Typo: P.12 L.14 which is import for --> which is important for
2) P.13.L. 12 we will determine --> we determined
Response：We have corrected all the typos/errors in the manuscript.

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1. The function of BGL3 in protecting cells from DNA damage is a major finding and should be better supported by using at least two cell lines per assay.
Response：We performed the suggested experiments using three cell lines (HCT116, MCF7 and MDA-MB-231). Results were consistent with our earlier findings. As shown in Fig. 2C-2E and EV3D-3F, BGL3 protects cells from DNA damage.

In addition, it is not clear if BGL3 deficient cells are dying from DNA damage or just arrest. The manuscript will benefit from more thorough description of the consequence of BGL3 depletion and DNA damage to cell survival.
Response：As shown in Fig EV4D, knockdown of BGL3 promoted cell death, not just arrest. We have included these results in the revised version.

2. The authors use siRNA to manipulate BGL3 and, for example, follow the recruitment of various proteins to damage sites within the nucleus. siRNA function by depleting the cytosolic RNA pool leading to the question of where is the damage site localized BGL3 RNA coming from? This question is made more relevant by the finding that BGL3 expression is not induced by damage and therefore, most likely, recruited BGL3 is 'old RNA'. One way to address this question is fractionate the cell into cytosolic, nuclear and chromatin fractions and probe for BGL3 before and following DNA damage.
Response： As shown in FigEV3C, DNA damage induced BGL3 shuttling from cytoplasm to the nucleus and promoted its binding to chromatin. These results support our model.

Henning W, Sturzbecher HW (2003) Homologous recombination and cell cycle checkpoints: Rad51 in tumour progression and therapy resistance. *Toxicology* **193:** 91-109

Klein HL (2008) The consequences of Rad51 overexpression for normal and tumor cells. DNA repair 7: 686-693

Lee JS, Collins KM, Brown AL, Lee CH, Chung JH (2000) hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 404: 201-204

Li J, Han L, Roebuck P, Diao L, Liu L, Yuan Y, Weinstein JN, Liang H (2015) TANRIC: An

Interactive Open Platform to Explore the Function of lncRNAs in Cancer. Cancer research 75: 3728-3737

Zhang H, Liu H, Chen Y, Yang X, Wang P, Liu T, Deng M, Qin B, Correia C, Lee S, Kim J, Sparks M, Nair AA, Evans DL, Kalari KR, Zhang P, Wang L, You Z, Kaufmann SH, Lou Z, Pei H (2016) A cell cycle-dependent BRCA1-UHRF1 cascade regulates DNA double-strand break repair pathway choice. Nature communications 7: 10201

2nd Editorial Decision 24th March 2020

Thank you again for submitting your revised manuscript to The EMBO Journal, and for your clarification of authorship details. Two of the original referees have now assessed the new version and your responses once more (see below), and I am pleased to say have no further scientific concerns. Following textual revision of a remaining minor point of referee 3, as well as incorporation of the below-listed formal/editorial issues, we shall therefore be happy to accept the manuscript for publication.

REFEREE REPORTS

Referee #1:

The authors have done a fine job addressing my points and revising the manuscript.

I have no other issue.

Referee #3:

The authors addressed my concerns adequately. Regarding my original concern bellow and the authors' response to it, the authors should explain how they measured cell death:

>"In addition, it is not clear if BGL3 deficient cells are dying from DNA damage or just arrest. The manuscript will benefit from more thorough description of the consequence of BGL3 depletion and DNA damage to cell survival."
>>"Response: As shown in Fig EV4D, knockdown of BGL3 promoted cell death, not just arrest. We have included these results in the revised version."

2nd Revision - authors' response 26th March 2020

We thank the referees for their positive comments on our revised manuscript.

Referee #1:

The authors have done a fine job addressing my points and revising the manuscript.

I have no other issue.
Response: We thank the reviewer for his/her support of our work.

Referee #3:

The authors addressed my concerns adequately. Regarding my original concern bellow and the authors' response to it, the authors should explain how they measured cell death:

>"In addition, it is not clear if BGL3 deficient cells are dying from DNA damage or just arrest. The manuscript will benefit from more thorough description of the consequence of BGL3 depletion and DNA damage to cell survival."
>>"Response: As shown in Fig EV4D, knockdown of BGL3 promoted cell death, not just arrest. We have included these results in the revised version."
Response: We used the Trypan Blue dye exclusion test to measure cell death. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not. In this test, a cell suspension is simply mixed with dye and examined under a microscopy to measure cell death.

Accepted 27th March 2020

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Huadong Pei & Chenxi Jia
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2019-104133

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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?	Samples sizes were chosen based on literatures and our previous studies.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples 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.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
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.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes, all statistical tests used are described in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	yes, For n<4 experiments, no statistical method was used to assess it. The assumptions were assesed based on literatures and our previous study.
Is there an estimate of variation within each group of data?	Yes. Error bar was used to show varation.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
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http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

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

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http://figshare.com

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

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

Is the variance similar between the groups that are being statistically compared?	Yes. Or we used tests for unequal variances.

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).	Citation and/or catalog number have been provided for all antibodies used in this study in methods and materials.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Reported in Materials and Methods, "Cell lines" subsection. These cell lines were all characterized by DNA finger printing analysis and passaged less than 6 months in this study. Cells were tested for mycoplasma prior to use for experiments.

* 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.	NA
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.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The mass spectrometry data from this publication have been deposited to the PRIDE database http://www.ebi.ac.uk/pride/archive/projects/PXD018186 and assigned the identifier PXD018186.
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).	yes,All important data deposited.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No, it does not.