EMBO reports

DOCK5 Regulates Energy Balance and Hepatic Insulin Sensitivity by Targeting mTORC1 Signaling

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Review timeline:

Submission date:	14 October 2019
Editorial Decision:	7 November 2019
Revision received:	14 November 2019
Accepted:	29 November 2019

Editor: Deniz Senyilmaz-Tiebe

Transaction Report:
Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at EMBO Reports

(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 7 November 2019

Thank you for submitting your manuscript to EMBO Reports, which has been reviewed at another journal. I have now carefully assessed your revised manuscript and received the report of the arbitrating advisor.

As you can see, the advisor finds that the study is significantly improved during revision and recommends publication here. Before I can accept the manuscript, I need you to address some minor points below:

Review - 1

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This manuscript entitled “DOCK5 Regulates Energy Balance and Hepatic Insulin Sensitivity 1 by Targeting mTOC1 Signaling” by Dr. Gangyi Yang’s group. Although the dedicator of cytokinesis 5(DOCK5) has been shown to be linked to obesity in humans, the underlying mechanisms are largely unknown. This manuscript presented that DOCK5 deficiency resulted in decreased energy expenditure, increased adiposity, and glucose intolerance in HFD-fed mice. Furthermore, mTORC1 mediated the metabolic effect of DOCK5 in diet-induced insulin resistant mice. Authors also showed that liver-specific knockout of Raptor diminished the ability of DOCK5 deficiency to enhance insulin resistance during obesity. The description of mTORC1 pathway responsible for the effect of DOCK5 is an important gain of knowledge. The experiments are well designed and the results are very interesting.

There are some concerns and suggestions:

Although Fig. 1 showed that DOCK5 regulates energy expenditure, it is unclear which mechanism (s) are involved in adipose tissue's functions. Please discuss it in the text.

In Fig. 3A, it seems unclear why hepatic G6Pase and PEPCK expression was not altered in HFD-fed WT mice, which was inconsistent with increased HGP in HFD-fed WT mice (Fig. 2F).

In Fig. 5, the experimental design has been described as "Raptor fl/fl male mice (8 weeks) were fed a HFD for 12 weeks and injected with AAV8-T-GFP 683 or AAV8-T-shDOCK 5, or AAV8-T-shDOCK5 + AAV8-T-Cre via tail vein." It is unclear whether Raptor fl/fl mice should be Raptor fl/fl Cre+ mice, but not Raptor fl/fl mice. How did authors perform AAV injection in HFD-fed mice? Authors should include a detailed protocol of these studies and also show the in vivo knockout of Raptor and knockdown of DOCK5 in the liver of mice.

It is unclear whether other DOCK family has similar metabolic effects on diabetes and obesity.

It would be interesting to include more background and discuss how DOCK5 functions as Rho GTPase signaling to regulate mTORC1 kinase.

Reviewer #2 (Remarks to the Author):

In this manuscript, Lai Y. et al. investigated the mechanistic basis by which Dock5 expression levels contribute to general metabolism in mice. Genetic inactivation of Dock5 altered a number of metabolic parameters especially under high fat diet conditions. In particular, the mechanistic studies suggest that there is an activation of one branch of the mTOR pathway upon deletion of Dock5. Furthermore, Dock5 deletion or its over-expression changed the expression levels of components of mTOR signaling (e.g. Raptor). An experiment carried out in hepatic Raptor-cKO conditions suggest that the effects of Dock5 depletion on metabolism are eliminated, which is in contrast to control conditions. The authors also show a potentially direct interactions between Dock5/Raptor. In summary, they conclude that Dock5 negatively regulates the activity of the Raptor branch of the mTOR pathway to control global metabolism.

The storyline is supported with multiple in vivo experiments that strengthen the link between Dock5 and global metabolism. However, the mechanistic studies remain poorly developed such that we have no new or clear insights of how Dock5 signals.

Major comments

1. The authors describe some convincing changes in expression levels or activity levels of AKT/mTOR components when Dock5 is eliminated. However, this works remains largely descriptive and fails to address how Dock5 mediates these changes. The link between Dock5 and mTOR brings a novel interaction between Raptor and Dock5 (although this is in overexpression conditions and not further confirmed on endogenous proteins).

Q1-Dock5 is a very specific GEF for Rac1 GTPases and this is the major function of this protein. Does DOCK5 uses its GEF activity to inhibit the mTOR pathway? Can the author carry out o/e experiments with WT and GEF dead Dock5 and test the impact on mTOR activity?

Q2-There is a major report of a direct interaction between Dock5 and AKT (Ogawa K. et al. 2014 JEM) – while this reference is cited the authors don't discuss the link to AKT.

Q3-Is the deletion of Dock5 leading to decreased Rac1 GTP-loading and this is what activates mTOR? Can Dock5 depleted cells be rescued with active Rac1 to decrease mTOR activity? This level of understanding is required to provide impactful mechanistic information.

2. The interaction between Raptor and Dock5 needs to be further demonstrated. Can the authors map out the interreacting regions? Does abrogating the Dock5/Raptor interaction leads to increased mTOR signaling?

Additional comments

1. Reading of the material and methods revealed that the authors did not generate the Dock5-null mice (in contrast to what is stated in the result section: …DOCK5 knockout mice were generated). In the methods, the authors refer to reference 7 for the generation of this mouse line (which, upon reading, is incorrect as this paper refers Laurin M. et al. PNAS 2008). This should be corrected.

2. The discussion section is long and repeats many of the results. It should focus on the main findings and position them in the field. The introduction does not prepare well for the reading of the paper – the authors should consider introducing the mTOR pathway, AKT etc. and their key roles in metabolism.

Authors' point-by-point response-1

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This manuscript entitled "DOCK5 Regulates Energy Balance and Hepatic Insulin Sensitivity 1 by Targeting mTOC1 Signaling" by Dr. Gangyi Yang's group. Although the dedicator of cytokinesis 5(DOCK5) has been shown to be linked to obesity in humans, the underlying mechanisms are largely unknown. This manuscript presented that DOCK5 deficiency resulted in decreased energy expenditure, increased adiposity, and glucose intolerance in HFD-fed mice. Furthermore, mTORC1 mediated the metabolic effect of DOCK5 in diet-induced insulin resistant mice. Authors also showed that liver-specific knockout of Raptor diminished the ability of DOCK5 deficiency to enhance insulin resistance during obesity. The description of mTORC1 pathway responsible for the effect of DOCK5 is an important gain of knowledge. The experiments are well designed, and the results are very interesting.

There are some concerns and suggestions:

1. Although Fig. 1 showed that DOCK5 regulates energy expenditure, it is unclear which mechanism (s) are involved in adipose tissue's functions. Please discuss it in the text.
Response: To address this comment, UCP1 mRNA expression has been measured in the brown adipose tissue (BAT) of DOCK5$^{-/-}$ and WT mice, and as requested, this content has been discussed (See Page 7, 16 and Figure S2, Table S4 and Ref. 17).

2. In Fig. 3A, it seems unclear why hepatic G6Pase and PEPCK expression was not altered in HFD-fed WT mice, which was inconsistent with increased HGP in HFD-fed WT mice (Fig. 2F).
Response: Fig. 3A has been carefully examined. In HFD- and SD-fed WT mice, the expression of PEPCK and G6Pase mRNA has been compared. The levels of PEPCK and G6Pase protein have been re-measured by Western bolts and compared in HFD- and SD-fed WT mice (See Fig. 3A).

3. In Fig. 5, the experimental design has been described as "Raptor$^{fl/fl}$ male mice (8 weeks) were fed a HFD for 12 weeks and injected with AAV8-T-GFP or AAV8-T-shDOCK5, or AAV8-T-shDOCK5 + AAV8-T-Cre via the tail vein." It is unclear whether Raptor$^{fl/fl}$ mice should be Raptor$^{fl/fl}$ Cre+ mice, but not Raptor$^{fl/fl}$ mice. How did authors perform AAV injection in HFD-fed mice? Authors should include a detailed protocol of these studies and also show the in vivo knockout of Raptor and knockdown of DOCK5 in the liver of mice.
Response: Raptor$^{fl/fl}$ mice have been corrected based on reviewer 1's comments and other publications (1-3) (Figure 5 and page 12). As requested, a detailed protocol for AAV injection in HFD-fed Raptor$^{flox/flox}$ mice has been shown in Fig. 5a and further described in the Materials and Methods and Figure 5 legends (See page 23 and 39). In addition, knockout of Raptor and knockdown of DOCK5 in the liver of mice have been shown in Fig. 6c, and Fig. S8 (See Figure Legends for Fig. 6c).

References

1) Cyclophilin D deficiency attenuates mitochondrial perturbation and ameliorates hepatic steatosis. Hepatology. 2018; 68: 62-77.
2) Hepatic CREBZF couples insulin to lipogenesis by inhibiting insig activity and contributes to h epatic steatosis in diet-induced insulin-resistant mice.Hepatology. 2018;68:1361- 1375.
3) Hepatocyte-Specific β-Catenin Deletion During Severe Liver Injury Provokes Cholangiocytes to Differentiate Into He patocytes. Hepatology. 2019; 69: 742-759.

4. It is unclear whether other DOCK family has similar metabolic effects on diabetes and obesity.
Response: As requested, the effect of DOCK2 on obesity has been added to the Introduction (See Page 5). In addition, we failed to find that other members of the DOCK family had similar metabolic effects on obesity and diabetes.

5. *It would be interesting to include more background and discuss how DOCK5 functions as Rho GTPase signaling to regulate mTORC1 kinase.*
Response: As requested, the levels of Rac GEF activity in MPHs from WT and DOCK5$^{-/-}$ mice have been examined (See page 14 and Figure 7a). Overexpression experiment with WT and GEF dead Dock5 has been performed *in vitro,* and the impact of WT and mutant DOCK5 on mTOR activity has also been observed. These contents have been further discussed (See page 19).

Reviewer #2

In this manuscript, Lai Y. et al. investigated the mechanistic basis by which Dock5 expression levels contribute to general metabolism in mice. Genetic inactivation of Dock5 altered a number of metabolic parameters especially under high fat diet conditions. In particular, the mechanistic studies suggest that there is an activation of one branch of the mTOR pathway upon deletion of Dock5. Furthermore, Dock5 deletion or its over-expression changed. The storyline is supported with multiple in vivo experiments that strengthen the link between Dock5 and global metabolism. However, the mechanistic studies remain poorly developed such that we have no new or clear insights of how Dock5 signals.

Major comments

Q1-Dock5 is a very specific GEF for Rac1 GTPases and this is the major function of this protein. Does DOCK5 use its GEF activity to inhibit the mTOR pathway? Can the author carry out o/e experiments with WT and GEF dead Dock5 and test the impact on mTOR activity?
Response: As requested, we first examined the levels of Rac GEF activity in MPHs from WT and DOCK5$^{-/-}$ mice (See page 14 and Figure 7a and b). We have performed overexpression experiments with WT and GEF dead Dock5 and observed the impact of DOCK5 on mTOR activity in hepa1-6 cells. In addition, the corresponding literature was cited (See page 14, 19, 22, and 24, Figure 7b-c and References 3, 10, 45-46).

Q2-There is a major report of a direct interaction between Dock5 and AKT (Ogawa K. et al. 2014 JEM) – while this reference is cited the authors don't discuss the link to AKT.
Response: As requested, the content has been added to the Discussion (See Page 17 and 19).

Q3-Is the deletion of Dock5 leading to decreased Rac1 GTP-loading and this is what activates mTOR? Can Dock5 depleted cells be rescued with active Rac1 to decrease mTOR activity?
Response: We thank the reviewer for this excellent comment. Rac1 GTP-loading has been measured and added to the Results (See page 14, and Figure 7a). In addition, we used SEW2871(a Rac activator) treatment to activate Rac1in MPHs from DOCK5$^{-/-}$ mice. The results showed that Rac1 activation in Dock5 depleted cells decreased mTOR activity (See page 14 and 24, and Figure 7b).

Q4-. The interaction between Raptor and Dock5 needs to be further demonstrated. Can the authors map out the interreacting regions? Does abrogating the Dock5/Raptor interaction leads to increased mTOR signaling?

Response: As requested, we further examined the interaction region between DOCK5 and Raptor. The localization of the DOCK5 binding site on Raptor was in the segment between amino acids 445– 887 (See page 15, 20, 22, 25, and Figure 7e).

To investigate whether DOCK5 interacts with Raptor through its DOCK homology region 2 (DHR-2) domain, we coexpressed GEF-dead DOCK5 (mutant DHR-2) with a Myc-tagged fragment (445-887 aa) of Raptor in Hepa1-6 cells. Indeed, the interaction between DOCK5 and Raptor (445-887 aa) was eliminated by the DHR-2 mutation of DOCK5 (Figure 7f). In addition, mTOR phosphorylation was increased in GEF-dead DOCK5 treated cells (Figure 7c). Therefore, it seemed likely that the DHR-2 domain of DOCK5 binds to Raptor (445-887aa) to regulate mTOR activity, which is mediated by the inhibition of Rac1 activation. These contents have been added to the MS (See page 15 and 20, and Figure 7f).

Additional comments

1. Reading of the material and methods revealed that the authors did not generate the Dock5-null mice (in contrast to what is stated in the result section: ...DOCK5 knockout mice were generated). In the methods, the authors refer to reference 7 for the generation of this mouse line (which, upon reading, is incorrect as this paper refers Laurin M. et al. PNAS 2008). This should be corrected.

Response: As requested, reference (Laurin M. et al. PNAS 2008) has been added to the MS (See page 22 and Ref. 37).

2. The discussion section is long and repeats many of the results. It should focus on the main findings and position them in the field. The introduction does not prepare well for the reading of the paper – the authors should consider introducing the mTOR pathway, AKT etc. and their key roles in metabolism.

Response: According to the comments of two reviewers, the discussion section has been revised or rewritten (See Discussion). As requested, the mTOR- Akt pathway has been introduced (See page 5).

Review - 2

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

All comments were addressed sufficiently.

Reviewer #2 (Remarks to the Author):

As stated previously, this is a potentially interesting manuscript that is missing some clear mechanistic insights into how DOCK5 interplays with the mTORC1 complex.

The author provide evidence for a direct interaction between Raptor and the DHR2 domain of DOCK5.

Unfortunately, the mechanistic data is far from convincing. The new results in Figure 7b and 7c are weak. I am not able to see the difference, as claimed by the author, in mTOR activation (pmTOR)? Specifically, the SEW2871 does not seem to have an effect (7B). Also, the expression of WT or GEF dead DOCK seems to have no impact (7C). It does not help that the data is not quantified.

There is also a disconnect with Ref 16 showing that the interaction of Rac1 signalling with mTorc1 or mTorc2 complexes is independent of its GDP/GTP loading state. How do the author reconstitute their data in line with this major report on the similar topic?

As such, a clear mechanism whereby DOCK5 impacts on mTOR signalling remains be demonstrated.

Authors' point-by-point response-2

Reviewers' comments:

Reviewer #1 (Remarks to the Author):
All comments were addressed sufficiently.
Response: Thanks.

Reviewer #2 (Remarks to the Author):
As stated previously, this is a potentially interesting manuscript that is missing some clear mechanistic insights into how DOCK5 interplays with the mTORC1 complex.
The author provide evidence for a direct interaction between Raptor and the DHR2 domain of DOCK5.

1. *Unfortunately, the mechanistic data is far from convincing. The new results in Figure 7b and 7c are weak. I am not able to see the difference, as claimed by the author, in mTOR activation (pmTOR)? Specifically, the SEW2871 does not seem to have an effect (7B). Also, the expression of WT or GEF dead DOCK seems to have no impact (7C). It does not help that the data is not quantified.*

Response: In Figures 7B and C, mTOR/p-mTOR have been replaced by another WB bands from four repeated experiments, and the data have been quantified by a gray analysis (See Figure 7B and C, and Figure Legends). In addition, Figures 7B and C have been identified by two molecular biologists and are considered to be significantly different. Therefore, we believe that these results fully demonstrate the existence of DOCK5-Raptor interaction.

2. *There is also a disconnect with Ref 16 showing that the interaction of Rac1 signalling with mTorc1 or mTorc2 complexes is independent of its GDP/GTP loading state. How do the author reconstitute their data in line with this major report on the similar topic?*

As such, a clear mechanism whereby DOCK5 impacts on mTOR signalling remains be demonstrated.

Response: We thank Reviewer 2 for this comment. At present, whether Rac1 signal interacts with mTOR complex depends on its GDP / GTP loading state is still controversial (Cancer Res. 2011; 71: 3246; Biochem Biophys Res Commun. 2008; 368: 132; Kobe J Med Sci. 2018; 64: E200; Mol Cell. 2011; 42: 50). Therefore, we stated this content and cited these documents (See page 19, Ref 16, 35-37).

Arbitrating advisor of *EMBO Reports*:

This is paper provides convincing evidence that DOCK5 stimulates mTORC1 activity through RAC GTPase and that through a well characterised feedback pathway operating through IRS1 and Akt controls hepatic insulin sensitivity. The data is robust making use DOCK5 KO mice models coupled with state-of-the-art approaches to monitor signalling pathways and insulin sensitivity gene expression and gluconeogenic analysis {plus minus} high fat diet treatment. The experiments showing that knock-down of raptor inhibits DOCK5 from controlling gluconeogenic gene expression supports the model. The authors present reasonable evidence that GEF activity of DOCK5 and activation of Rac lie behind the ability of DOCK5 to activate mTORC1. The mechanism by which Rac1 might activate mTORC1 is currently unknown and would require further analysis to understand. I think this study is suitable for EMBO reports without this data and will pave the way for further work to understand how Rac may control mTORC1. The paper is clearly written. Figures are also clear and well presented. Overall I believe this study makes a useful addition to the literature.

1st Revision - authors' response 14 November 2019

The authors performed all minor editorial changes.

2nd Editorial Decision 29 November 2019

Thank you for submitting your revised manuscript. I have now looked at everything carefully and all looks fine. Therefore I am very pleased to accept your manuscript for publication in EMBO Reports.

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: Ling Li, Gangyi Yang
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-49473

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 justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

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

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

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

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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?	Experiments were performed using sample sizes based on standard protocols in the field. No statistical test was performed to predetermine sample size.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Experiments were performed using sample sizes based on standard protocols in the field. No statistical test was performed to predetermine sample size.
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 study
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.	All mice were randomly allocated to the indicated groups.
For animal studies, include a statement about randomization even if no randomization was used.	All mice were randomly allocated to the indicated groups.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Blinding was used for analysis of all animal experimends expect qRT-PCR and immunoblot
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding was used for analysis of all animal experimends expect qRT-PCR and immunoblot
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA

Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

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).	"Methods">"mRNA and Protein Analysis"
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	"Methods">"Cell culture and treatment"

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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.	"Methods">"Animals and treatments"
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All experimental procedures were approved by the Animal Experimentation Ethics Committee,Chongqing Medical University（"Methods">Animals and treatments")
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.	Yes,we confirmed.

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	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. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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