EMBO reports

YTHDF1-Mediated Translation Amplifies Wnt-Driven Intestinal Stemness

Bing Han, Sujun Yan, Saisai Wei, Jie Xiang, Kangli Liu, Zhanghui Chen, Rongpan Bai, Jinghao Sheng, Zhengping Xu, and Xiangwei Gao

Review timeline:

Submission date:	16 September 2019
Editorial Decision:	6 October 2019
Revision received:	21 November 2019
Editorial Decision:	18 December 2019
Revision received:	23 December 2019
Editorial Decision:	10 January 2020
Revision received:	11 January 2020
Accepted:	15 January 2020

Editor: Achim Breiling

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 6 October 2019

Thank you for the submission of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to assess the manuscript, which can be found at the end of this email.

As you will see, all referees think that the findings are of high interest, but they also have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication in EMBO reports. As the reports are below, and I think all points need to be addressed, I will not detail them here.

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

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact me if a 3-months time frame is not sufficient so that we can discuss the revisions further.

When submitting your revised manuscript, please also carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision. When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that the changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#manuscriptpreparation

See also our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that ALL primary datasets produced in this study (e.g. RNA-seq. data) are deposited in an appropriate public database. See: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

6) We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

7) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

8) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

9) Please format the references according to our journal style. See:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

Finally, please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

Han et al use a combination of in vitro, ex vivo and in vivo platforms to investigate the role of YTHDF1 during intestinal homeostasis, regeneration and tumour initiation/growth. Overall the work is well presented, well written, logically approached and presents a convincing role of YTHDF1 as a regulator of intestinal regeneration and tumourigenesis via translational control of several Wnt pathway components. These data establish YTHDF1 as an important regulator of the Wnt pathway and an attractive therapeutic target for cancers with high Wnt.

One major concern is the conflicting data in Fig EV1F and 1G. The authors correctly highlight that beta-catenin is mutated in the AOM/DSS model. However, in the AOM/DSS model the authors show that tumourigenesis is reduced in YTHDF1 mutant mice. Similarly data in EV3I-K shows YTHDF1 is required for Lgr5 expression and sphere formation in HCT116 cells which have mutant beta-catenin. These data suggest that YTHDF1 is downstream of beta-catenin in the Wnt pathway however figure EV1F and G show that non-degradable beta-catenin does not regulate YTHDF1. As YTHFD1 is not important in normal ISCs but rather is upregulated in transformed intestinal cells, how do the authors propose YTHFD1 is being activated in HCT116 cells or AOM/DSS treated mice which are driven by mutant beta-catenin, as the data in EV1F and G show YTHFD1 levels do not respond to non-degradible beta-catenin? Can the authors please explain these seemingly conflicting

observations? For example how do the authors propose YTHDF1 is being regulated in mutant APC cells, as these would have deregulated (albeit not mutant) beta-catenin? Indeed Fig EV1F and G suggest the title of the manuscript should be changed to Wnt driven instead of beta-catenin driven.

Please see specific minor concerns below:

1. Can the authors please expand on how they treatment mice with Wnt3a and include it in the materials and methods in fig 1.

2. The authors show in Fig 1C that increased APC, and subsequent reduced beta-catenin, result in reduced protein levels of YTHDF1. Could the authors please expand on this observation - do they think

3. Could the authors please perform some analysis of Wnt target genes in the intestine of their irradiate YTHDF1 wt and mutant mice in Fig 2(including Myc, Lgr5 and Fzd7 to be consistent with some of the data already included in the manuscript) to determine if YTHDF1 is regulating Wnt signalling during intestinal regeneration.

4. Can the authors please perform statistical analysis on the data in 4E and F and present it on the graphs?

5. Fig 5 shows that YTHDF1 regulates components of the Wnt pathway. Could the authors please expand on this in the discussion (and include the references below) as Fzd7 in particular has recently been shown to regulate Lgr5 cells in the intestine (Flanagan et al Stem Cell Reports, 2015), to control intestinal regeneration via Myc. Indeed Myc is critical for intestinal regeneration (Ashton et al Dev Cell, 2010), and not just a marker of proliferation as suggested in the results section, thus these data and the data in Fig 2C are consistent with other models proposed in the field. Indeed many of the phenotypes described (stem cell function, regeneration and Wnt signalling activity) can be attributed to YTHDF1 regulation of Fzd7 as shown in Fig 5F which should be mentioned in the discussion please.

6. Can the authors please include a description of the data in Fig 5F in the figure legend and also add analysis of Lgr5 here as Lgr5 is not included in any of the Supplemental tables investigating YTHDF1-regulated mRNAs which seems odd given that it has been the subject of the investigations elsewhere in the manuscript?

7. Can the authors please refer to Fig 5F instead of Fig 5E in the sentence, 'Focusing on the potential candidates implicated in Wnt signaling, we noticed that T-cell factor 7 like 2/T-cell factor 4 (TCF7L2/TCF4) was listed as the top candidate, showing reduced translation upon YTHDF1 deletion (Figures 5E and EV4G)'.

8. Fig 7E shows that lifespan is increased in Min mice with deleted YTHDF1 - can the authors please include the data associated with this observation regarding the number and size of the intestinal tumours in these cohorts of Min mice?

9. Can the authors please perform some analysis on the tumours from the Lgr5Cre; YTHDF1fl/fl AOM/DSS or Min cohorts in Fig 7 for TCF, Myc and FZd7 to confirm if the same mechanism described in the previous experiments is responsible for the exciting observations in fig 7?

10. Although the athours show convincing data that TCF7L2 is a key target of YTHFD1 in regulating Wnt signalling, can the authors please also highlight in the discussion that other genes they have identified as YTHFD1 targets, specifically those in Fig 5F including Fzd7 and Myc, could also be important functional targets of YTHFD1 in regulating intestinal regeneration and cancer.

Referee #2:

The paper entitled « m6A-YTHD1-mediated translation amplifies b-catenin-driven intestinal stemness » describes the impact of the methylation m6A reader YTHDF1 on gut, including normal

homeostasis, regeneration as well as intestinal tumorigenesis. The authors nicely show that YTHDF1 acts as an amplifier of the Wnt/b-catenin pathway signaling. Overall this study is very original, well written and described, authors are using most often relevant models to address their questions and I particularly appreciated the graphical abstract and the illustrated protocols performed on mice. However I have 3 major points that need to be answered:

1) First, I don't think that you can say that all of your effects are mediated through m6A. To do so you will need to show the real localization of YTHDF1 / m6A on their targeted transcripts. The analysis is too global to conclude anything since different m6A sites could have opposite effects and could be recognized by different readers. Moreover authors show high translational modifications which could impact non direct readers of m6A. This means that a CLIP-seq should be performed or at least CLIP-qPCR on selected targeted transcripts (CLIP-qPCR : Method Mol Biol 2016, YoonJH, Gorospe). Authors should also performed in vitro knock down of METTL3 (the writer) to validate that described effects are indeed mediated by m6A. Finally m6A methylation should be monitored at least in the key experiments, this is now feasible by mass spectometry. If authors cannot perform these experiments they should remove m6A from the tittle.

2) Secondly, I think that the paragraph related to the cancer stem cell phenotype should be removed since it is not mandatory in the present story and results described in figure 4G and 4H are not definitely showing that you impact on this subpopulation of cancer cells. To my knowledge LGR5 is not a recognized cancer stem cell marker, the percentage of CSC are impossibly high within cancer cells and especially in SW620 which is not recognized as a cell line with high CSC phenotype. To ensure that the effect on spheres is related to CSC and not progenitors authors need to pass several times their spheres and validate that the effect is lasting over the passages. Finally the gold standard experiment to prove an impact on CSC is the tumorigenicity in vivo by injecting low dose of cells (ELDA in vivo).
3) I might have missed something but for me there is a lack between APC and YTHDF1, why its expression is upregulated when APC is mutated. Is it a direct Wnt/b-catenin target gene which in turn will amplify the pathway? This point has to be better explained.

Minor Points:
4) TCGA data could strengthen the paper since rapid researches seem to suggest that YTHDF1 is highly expressed in colorectal tumors (highest expression in the epitranscriptomic family).
5) Quantification analysis need to be added on figure 1A because to my eyes the upregulation of YTHDF1 expression is not that high and METTL3 one is not obvious neither.
6) On Ythdf1 KO mouse intestine is there a default of differentiation? All cell types are similarly represented? It has been surely studied please add a sentence in the text.
7) At the end of the paragraph "YTHDF1 is required for maintenance of mouse intestinal stem cells" authors should remove the last sentence (these results indicated an m6A dependent role of YTHDF1 in ISCs maintenance because it is wrong.
8) Figure 4G it is written CD44v6 and in the text it is CD44.
9) Errors in the legend: analysis of ontologies are not mentioned and heatmap is written in figure 5E whereas it is 5F

Referee #3:

This is a quite intriguing work further highlighting the crucial roles of YTHDF1 and m6A in tumorigenesis and stemness maintenance of specific stem cells. The authors have presented compelling evidence showing that Wnt-signaling components are able to regulate mRNA translational efficiency of Ythdf1 in both mouse intestine and human CRC cells. Genetic disruption of Ythdf1 leads to retardation of Wnt-driven intestinal regeneration and tumorigenesis. Further, consistent with its high expression in intestinal crypts, YTHDF1 was found to be essential for maintaining the stemness of both ISC and CRC stem cells. The characterization of YTHDF1 in multiple models make the physiological evidence quite convincing. Mechanistically, they presented a novel regulatory mechanism of the Tcf1 expression involving YTHDF1-m6A mediated Tcf4 mRNA translation during the activation of Wnt signaling pathway. This study should have significant implications on colorectal cancer therapeutics as well as epitranscriptomic studies.

Specific points:

(1) Based on the polysome curves (Fig. 1B and 1D), it seems that Wnt signaling is capable to positively modulate global translation. It appears that both controls show quite different patterns of polysome fractions. Does this mean global translation in intestinal crypt is not as efficient as in SW620 cells?

(2) It is obvious that fold changes of YTHDF1 during activation (Wnt-3a treatment) or inactivation (APC overexpression) of Wnt signaling is much greater than that of luciferase reporter assay or ribosome profiling-coupled with qPCR. Does it mean that Wnt could regulate the expression of YTHDF1 at additional layer? A recent study revealed that amplified DNA copy number might contribute to the high expression of YTHDF1 in CRC cells (Front Oncol. 2019 May 3;9:332). Authors may discuss this alternative regulatory model. Further, it will be helpful to quantify the fold changes of YTHDF1 protein levels in western blots (Fig. 1A and 1C).

(3) The authors presented sufficient evidence showing that YTHDF1, acting downstream of APC, is essential for Wnt-activated physiological processes including regeneration and tumorigenesis, as silencing β-catenin did not affect YTHDF1 expression. However, YTHDF1 could be a positive regulator of β-catenin (Front Oncol. 2019 May 3;9:332). It will be nice to blot β-catenin in YTHDF1-depleted cells harboring active Wnt. If this is the case, they may consider the YTHDF1-β-catenin regulatory pathway in the proposed model (Fig. 7F).

(4) Based on the rescue experiments using YTHDF1 WT and mutants (Fig. 4E and 4F), Han et al. concluded that YTHDF1 played a m6A-dependent role of in ISCs maintenance. It means that YTHDF1 is able to facilitate translation of at least m6A-containing RNA. How to explain increased expression of differentiation marker genes in response to Ythdf1 depletion?

(5) Among the Wnt signaling components, authors selected Tcf7l2 as a target for further study, as it contains multiple m6A sites across 5'UTR, CDS and 3'UTR regions. It has been implied that YTHDF1 recognizes the m6As on 3'UTR and facilitate the recruitment of translation initiation factors, promoting the translation of its target mRNAs (Cell. 2015 Jun 4;161(6):1388-99). To further confirm that 'Wnt signaling regulates the translation of TCF7L2 in an m6A-dependent manner', it would be nice to see whether YTHDF1 could promote the translation of Tcf7l2 through m6a methylation in 3'UTR. Further, to clarify the importance of YTHDF1-m6A in the regulation of Tcf7l2 expression in Wnt signal transduction pathway, the protein levels of TCF7L2 could be detected in control and Ythdf1-depleted cells in the presence of active Wnt. These pieces of evidence would make the work more compelling, although not absolutely essential.

1st Revision - authors' response 21 November 2019

Referee #1:

Han et al use a combination of in vitro, ex vivo and in vivo platforms to investigate the role of YTHDF1 during intestinal homeostasis, regeneration and tumor initiation/growth. Overall the work is well presented, well written, logically approached and presents a convincing role of YTHDF1 as a regulator of intestinal regeneration and tumourigenesis via translational control of several Wnt pathway components. These data establish YTHDF1 as an important regulator of the Wnt pathway and an attractive therapeutic target for cancers with high Wnt.

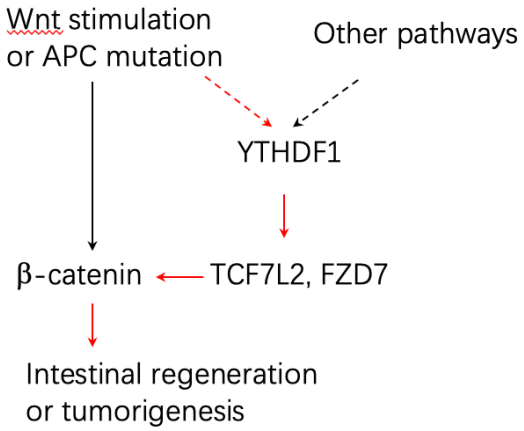
One major concern is the conflicting data in Fig EV1F and 1G. The authors correctly highlight that beta-catenin is mutated in the AOM/DSS model. However, in the AOM/DSS model the authors show that tumourigenesis is reduced in YTHDF1 mutant mice. Similarly data in EV3I-K shows YTHDF1 is required for Lgr5 expression and sphere formation in HCT116 cells which have mutant beta-catenin. These data suggest that YTHDF1 is downstream of beta-catenin in the Wnt pathway however figure EV1F and G show that non-degradable beta-catenin does not regulate YTHDF1. As YTHFD1 is not important in normal ISCs but rather is upregulated in transformed intestinal cells, how do the authors propose YTHFD1 is being activated in HCT116 cells or AOM/DSS treated mice which are driven by mutant beta-catenin, as the data in EV1F and G show YTHFD1 levels do not

respond to non-degradible beta-catenin? Can the authors please explain these seemingly conflicting observations? For example how do the authors propose YTHDF1 is being regulated in mutant APC cells, as these would have deregulated (albeit not mutant) beta-catenin? Indeed Fig EV1F and G suggest the title of the manuscript should be changed to Wnt driven instead of beta-catenin driven.
Response: The referee's concern is about two important questions: 1) How is YTHDF1 regulated by Wnt signaling? 2) What's the function of YTHDF1 in Wnt/β-catenin-mediated tumorigenesis?

Our data related to the first question include: 1) Treatment of normal intestinal epithelial cells with Wnt promotes YTHDF1 protein expression. 2) Re-expression of wild-type APC in APC-mutant cells decreases YTHDF1 protein expression. 3) However, knockdown of the non-degradable β-catenin does not affect YTHDF1 expression. Based on these data, we proposed that YTHDF1 expression is regulated by Wnt/APC but not β-catenin. These seemingly conflicting results could be explained by the published findings that Wnt/APC could regulate gene expression independent of β-catenin. For instance, Wnt was reported to regulate mRNA translation through mTOR (Inoki K. et al, Cell. 2006). Moreover, APC could regulate the activity of transcription factor YAP, potentially regulating gene expression (Cai J. et al, Genes Dev. 2015). The mechanism of Wnt/APC-regulated YTHDF1 expression needs further investigation. We have discussed this in detail in the revised manuscript.

It should also be noted that other layers of regulation also exist, including gene transcription, as demonstrated by other groups (Nishizawa Y. et al, Oncotarget. 2018). Therefore, it is reasonable to propose that YTHDF1 might be upregulated in HCT116 cancer cells and AOM/DSS model. Actually, we have detected the upregulation of YTHDF1 in AOM/DSS model (Figure EV1I).

As for the function of YTHDF1, our data demonstrated that: 1) YTHDF1 is required for efficient regeneration and tumorigenesis. 2) YTHDF1 is required for Wnt-driven stemness of cultured organoids. 3) Mechanistically, we demonstrated that YTHDF1 regulates the translation of *TCF7L2* and *FZD7*, which are major regulators of β-catenin. The relationship between Wnt, APC, YTHDF1, and β-catenin is illustrated in our proposed model (Figure 7G of the revised manuscript), which is also shown as follows (our findings are highlighted in red arrows). From this model, Wnt/APC is upstream of YTHDF1 and β-catenin, while YTHDF1 acts as an amplifier for β-catenin. Based on these, we agree with the referee to change our title to "YTHDF1-mediated translation amplifies Wnt-driven intestinal stemness".



Please see specific minor concerns below:

1. Can the authors please expand on how they treat mice with Wnt3a and include it in the materials and methods in fig 1.
Response: We apologize for the confusion here. In Figure 1, we treated the isolated crypts but not mice with Wnt3a. Briefly, mice were sacrificed. Crypts were isolated from the small intestine and treated with Wnt3a. We have clarified this in the materials and methods (crypt isolation, treatment, and culture).

2. The authors show in Fig 1C that increased APC, and subsequently reduced beta-catenin, result in reduced protein levels of YTHDF1. Could the authors please expand on this observation.
Response: As mentioned above (response to the major concern), our data suggested that YTHDF1 expression is regulated by Wnt and APC but not β-catenin. These results could be explained by the published findings that Wnt/APC could regulate gene expression independent of β-catenin. We have explained this in the discussion of the revised manuscript.

3. Could the authors please perform some analysis of Wnt target genes in the intestine of their irradiate YTHDF1 wt and mutant mice in Fig 2 (including Myc, Lgr5 and Fzd7 to be consistent with some of the data already included in the manuscript) to determine if YTHDF1 is regulating Wnt signalling during intestinal regeneration.
Response: We thank the referee for this suggestion. We determined the expressions of Wnt target genes including *Myc*, *Lgr5,* and *Fzd7* using qPCR. Data showed that knockout of *Ythdf1* reduced the expression of these genes during intestinal regeneration (Figure 2E).

4. Can the authors please perform statistical analysis on the data in 4E and F and present it on the graphs?
Response: Following the referee's suggestion, we performed statistical analysis on the data in Figure 4E and 4F and presented it on the revised graph.

5. Fig 5 shows that YTHDF1 regulates components of the Wnt pathway. Could the authors please expand on this in the discussion (and include the references below) as Fzd7 in particular has recently been shown to regulate Lgr5 cells in the intestine (Flanagan et al Stem Cell Reports, 2015), to control intestinal regeneration via Myc. Indeed Myc is critical for intestinal regeneration (Ashton et al Dev Cell, 2010), and not just a marker of proliferation as suggested in the results section, thus these data and the data in Fig 2C are consistent with other models proposed in the field. Indeed many of the phenotypes described (stem cell function, regeneration and Wnt signalling activity) can be attributed to YTHDF1 regulation of Fzd7 as shown in Fig 5F which should be mentioned in the discussion please.
Response: We greatly appreciate the referee's effort to improve our manuscript. Indeed, FZD7 has been demonstrated to be the major FZD receptor responsible for mediating Wnt activity in intestinal stem cells. In addition, FZD7 was reported to control intestinal regeneration via MYC (Flanagan et al Stem Cell Reports, 2015). Indeed, as a direct target of β-catenin, MYC has been shown to be critical for intestinal regeneration (Ashton et al Dev Cell, 2010). Importantly, our data strongly indicated the critical function of YTHDF1-regulated FZD7 expression in stem cell function, regeneration, and tumorigenesis. Therefore, we expanded our discussion on the roles of YTHDF1, FZD7, and MYC in intestinal regeneration. We have also included these references in the revised manuscript.

6. Can the authors please include a description of the data in Fig 5F in the figure legend and also add analysis of Lgr5 here as Lgr5 is not included in any of the Supplemental tables investigating YTHDF1-regulated mRNAs which seems odd given that it has been the subject of the investigations elsewhere in the manuscript?
Response: We agree with the referee that it would be valuable to include *LGR5* expression analysis in YTHDF1-deficient cells. However, because our sequencing data (m6A-seq, RIP, and Ribo-seq) did not catch *LGR5* gene, the information of this gene is not presented in any of the Supplemental tables. This is might due to the low expression level of this gene in HCT116 cells.

Since the data for Fig 5F (Fig 5E in the revised manuscript) is extracted from Ribo-seq data, we cannot add *LGR5* expression in this panel. However, our qPCR analysis showed that *LGR5* expression is dramatically reduced in YTHDF1-silenced HCT116 cells (Figure EV4H). As *LGR5* is a direct target of β-catenin (van de Wetering et al, Cell. 2002), our data, together with the luciferase assay (Figure 6G) strongly indicated reduced β-catenin activity after YTHDF1 knockdown.

7. Can the authors please refer to Fig 5F instead of Fig 5E in the sentence, 'Focusing on the potential candidates implicated in Wnt signaling, we noticed that T-cell factor 7 like 2/T-cell factor 4 (TCF7L2/TCF4) was listed as the top candidate, showing reduced translation upon YTHDF1 deletion (Figures 5E and EV4G)'.

Response: We are sorry for this error. Since the GO data has been presented in Table EV4, we deleted Figure 5E in the revised manuscript. Instead, we designated the heatmap panel as Figure 5E.

8. Fig 7E shows that lifespan is increased in Min mice with deleted YTHDF1 - can the authors please include the data associated with this observation regarding the number and size of the intestinal tumours in these cohorts of Min mice?
Response: We agree with the referee that it would be valuable if the number and size of tumors in the Min mice in Figure 7E (Figure 7F of the revised manuscript) are included. However, we cannot get the data because of the operating procedure. The lifespan was recorded only after the death of the mouse. Some mice bodies already became stiff and it was hard to isolate the tumors. However, knockout of *Ythdf1* using Vilin-Cre significantly reduced tumor number and size in Min mice (Figure 3C), demonstrating an essential role of epithelial YTHDF1 in *Apc* mutation-driven tumorigenesis.

9. Can the authors please perform some analysis on the tumours from the Lgr5Cre; YTHDF1fl/fl AOM/DSS or Min cohorts in Fig 7 for TCF, Myc and FZd7 to confirm if the same mechanism described in the previous experiments is responsible for the exciting observations in fig 7?
Response: Following the referee's suggestion, we detected the expressions of TCF7L2, MYC and FZD7 in the AOM/DSS treated WT and *Ythdf*-cKO mice. Data revealed the downregulation of these proteins in *Ythdf1*-cKO tumors compared to *Ythdf1*-WT tumors (Figure 7D).

10. Although the athours show convincing data that TCF7L2 is a key target of YTHFD1 in regulating Wnt signalling, can the authors please also highlight in the discussion that other genes they have identified as YTHFD1 targets, specifically those in Fig 5F including Fzd7 and Myc, could also be important functional targets of YTHFD1 in regulating intestinal regeneration and cancer.
Response: We agree with the referee that besides TCF7L2, other genes such as FZD7 could also be important functional targets of YTHDF1 in regulating intestinal regeneration and cancer. We have highlighted this in the discussion.

Referee #2:

The paper entitled « m6A-YTHD1-mediated translation amplifies b-catenin-driven intestinal stemness » describes the impact of the methylation m6A reader YTHDF1 on gut, including normal homeostasis, regeneration as well as intestinal tumorigenesis. The authors nicely show that YTHDF1 acts as an amplifier of the Wnt/b-catenin pathway signaling. Overall this study is very original, well written and described, authors are using most often relevant models to address their questions and I particularly appreciated the graphical abstract and the illustrated protocols performed on mice. However I have 3 major points that need to be answered:

1) First, I don't think that you can say that all of your effects are mediated through m6A. To do so you will need to show the real localization of YTHDF1/m6A on their targeted transcripts. The analysis is too global to conclude anything since different m6A sites could have opposite effects and could be recognized by different readers. Moreover authors show high translational modifications which could impact non direct readers of m6A. This means that a CLIP-seq should be performed or at least CLIP-qPCR on selected targeted transcripts (CLIP-qPCR : Method Mol Biol 2016, YoonJH, Gorospe). Authors should also performed in vitro knock down of METTL3 (the writer) to validate that described effects are indeed mediated by m6A. Finally m6A methylation should be monitored at least in the key experiments, this is now feasible by mass spectometry. If authors cannot perform these experiments they should remove m6A from the title.
Response: We agree with the referee that our conclusion is not accurately stated in the title. Using in vitro, ex vivo and in vivo platforms, we demonstrated an essential role of YTHDF1 in Wnt-driven intestinal stemness maintenance, regeneration, and tumorigenesis. Although knockdown of METTL3 reduces stemness in cultured organoids (Figures EV3D-G), the evidence is not strong enough to draw the conclusion that all the biological effects of YTHDF1 are mediated through m6A.

We agree that the sequencing analysis (m6A-seq, RIP-seq, and Ribo-seq) is only a hint but not a conclusion. We also agree with the referee that different m6A sites could have opposite effects. Following the referee's suggestion, we removed m6A from the title to make it more precise.

2) Secondly, I think that the paragraph related to the cancer stem cell phenotype should be removed since it is not mandatory in the present story and results described in figure 4G and 4H are not definitely showing that you impact on this subpopulation of cancer cells. To my knowledge LGR5 is not a recognized cancer stem cell marker, the percentage of CSC are impossibly high within cancer cells and especially in SW620 which is not recognized as a cell line with high CSC phenotype. To ensure that the effect on spheres is related to CSC and not progenitors authors need to pass several times their spheres and validate that the effect is lasting over the passages. Finally the gold standard experiment to prove an impact on CSC is the tumorigenicity in vivo by injecting low dose of cells (ELDA in vivo).
Response: We agree with the referee that the results related to the cancer stem cell growth are not mandatory to our present story since cancer stem cells are different from ISCs. Moreover, tumorspheres need to pass several times to validate the phenotype. Therefore, following the referee's suggestion, we removed figure 4G and 4H, and related results (Figures EV3H-EV3K), as well as the methods part of our manuscript.

3) I might have missed something but for me there is a lack between APC and YTHDF1, why its expression is upregulated when APC is mutated. Is it a direct Wnt/b-catenin target gene which in turn will amplify the pathway? This point has to be better explained.
Response: Our data indicated that YTHDF1 expression is regulated by Wnt/APC mutation but not β-catenin. This could be explained by the published findings that Wnt/APC could regulate gene expression independent of β-catenin. For instance, Wnt could regulate mRNA translation through mTOR (Inoki K. et al, Cell. 2006). Moreover, APC could regulate the activity of transcription factor YAP, potentially regulating gene expression (Cai J. et al, Genes Dev. 2015). The mechanism of Wnt/APC-regulated YTHDF1 expression needs further investigation. We have explained this in detail in the discussion part.

Minor Points:
4) TCGA data could strengthen the paper since rapid researches seem to suggest that YTHDF1 is highly expressed in colorectal tumors (highest expression in the epitranscriptomic family).
Response: We appreciate the referee's suggestion to use the TCGA data to evaluate YTHDF1 expression in CRC. Indeed, TCGA data showed that among the m6A-related proteins, YTHDF1 is the most dramatically upregulated in CRC tissues than adjacent normal tissues (Figure EV1L).

5) Quantification analysis need to be added on figure 1A because to my eyes the upregulation of YTHDF1 expression is not that high and METTL3 one is not obvious neither.
Response: Following the referee's suggestion, we quantified the fold changes of YTHDF1 protein levels in western blots and incorporated them into the revised Fig. 1A.

6) On Ythdf1 KO mouse intestine is there a default of differentiation? All cell types are similarly represented? It has been surely studied please add a sentence in the text.
Response: We have performed these studies and found that there is no significant default of differentiation in *Ythdf1* KO mouse intestine. Alkaline phosphatase staining (enterocytes) and Alcian blue staining (goblet cells) did not show significant difference between $Ythdf1^{CTL}$ and $Ythdf1^{cKO}$ intestine. We have incorporated these results in the revised manuscript (Figure EV2I).

7) At the end of the paragraph "YTHDF1 is required for maintenance of mouse intestinal stem cells" authors should remove the last sentence (these results indicated an m6A dependent role of YTHDF1 in ISCs maintenance because it is wrong.
Response: The point raised by the reviewer is valid. We have removed the last sentence "these results indicated an m6A dependent role of YTHDF1 in ISCs maintenance" in the revised manuscript.

8) Figure 4G it is written CD44v6 and in the text it is CD44.
Response: Following the referee's suggestion, we have deleted the results related to CD44v6.

9) Errors in the legend: analysis of ontologies are not mentioned and heatmap is written in figure 5E whereas it is 5F
Response: We are sorry for this error. Since the GO data has been presented in Table EV4, we deleted Figure 5E in the revised manuscript. Instead, we designated the heatmap panel as Figure 5E.

Referee #3:

This is a quite intriguing work further highlighting the crucial roles of YTHDF1 and m6A in tumorigenesis and stemness maintenance of specific stem cells. The authors have presented compelling evidence showing that Wnt-signaling components are able to regulate mRNA translational efficiency of Ythdf1 in both mouse intestine and human CRC cells. Genetic disruption of Ythdf1 leads to retardation of Wnt-driven intestinal regeneration and tumorigenesis. Further, consistent with its high expression in intestinal crypts, YTHDF1 was found to be essential for maintaining the stemness of both ISC and CRC stem cells. The characterization of YTHDF1 in multiple models make the physiological evidence quite convincing. Mechanistically, they presented a novel regulatory mechanism of the Tcf1 expression involving YTHDF1-m6A mediated Tcf4 mRNA translation during the activation of Wnt signaling pathway. This study should have significant implications on colorectal cancer therapeutics as well as epitranscriptomic studies.

Specific points:

(1) Based on the polysome curves (Fig. 1B and 1D), it seems that Wnt signaling is capable to positively modulate global translation. It appears that both controls show quite different patterns of polysome fractions. Does this mean global translation in intestinal crypt is not as efficient as in SW620 cells?
Response: Indeed, the polysome profiling data indicate that Wnt treatment promotes global translation, which is in line with the previous report that Wnt signaling activates mTOR to promote mRNA translation (Inoki K. et al, Cell. 2006). We discussed this in the revised manuscript.

Moreover, polysome profiles also indicated that the translation in isolated crypt (with relatively higher monosome and lower polysome) is not as efficient as in SW620 cells (relatively higher polysome).

(2) It is obvious that fold changes of YTHDF1 during activation (Wnt-3a treatment) or inactivation (APC overexpression) of Wnt signaling is much greater than that of luciferase reporter assay or ribosome profiling-coupled with qPCR. Does it mean that Wnt could regulate the expression of YTHDF1 at additional layer? A recent study revealed that amplified DNA copy number might contribute to the high expression of YTHDF1 in CRC cells (Front Oncol. 2019 May 3;9:332). Authors may discuss this alternative regulatory model. Further, it will be helpful to quantify the fold changes of YTHDF1 protein levels in western blots (Fig. 1A and 1C).
Response: We agree with the referee at the point that Wnt might regulate the expression of YTHDF1 at an additional layer. We have carefully discussed the regulation of YTHDF1 expression in the revised manuscript.

Following the referee's suggestion, we have quantified the fold changes of YTHDF1 protein levels in western blots and incorporated them into the revised Fig. 1A and 1C.

(3) The authors presented sufficient evidence showing that YTHDF1, acting downstream of APC, is essential for Wnt-activated physiological processes including regeneration and tumorigenesis, as silencing β-catenin did not affect YTHDF1 expression. However, YTHDF1 could be a positive regulator of β-catenin (Front Oncol. 2019 May 3;9:332). It will be nice to blot β-catenin in YTHDF1-depleted cells harboring active Wnt. If this is the case, they may consider the YTHDF1-β-catenin regulatory pathway in the proposed model (Fig. 7F).
Response: Following the referee's suggestion, we detected β-catenin expression in Ythdf1-depleted crypt during Wnt treatment and found that the protein level of β-catenin decreased in Ythdf1-deleted crypt (Figure EV5B). Our Ribo-seq data revealed no dramatic change for β-catenin translation. However, YTHDF1 could regulate the expression of Wnt signaling components such as FZD7 and DVL3, all of which are reported to regulate β-catenin protein stability. Therefore, we proposed that YTHDF1 might regulate the degradation of β-catenin through FZD7 or DVL3. Following the referee's suggestion, we have revised the proposed model (Figure 7G of the revised manuscript).

(4) Based on the rescue experiments using YTHDF1 WT and mutants (Fig. 4E and 4F), Han et al. concluded that YTHDF1 played a m6A-dependent role of in ISCs maintenance. It means that YTHDF1 is able to facilitate translation of at least m6A-containing RNA. How to explain increased expression of differentiation marker genes in response to Ythdf1 depletion?
Response: The increased expression of differentiation marker genes after Ythdf1 deletion should be an indirect effect. Ythdf1 depletion reduces β-catenin activity, leading to the differentiation of the cultured organoids. Since the differentiated cells increased, it is not surprising to see the upregulation of differentiation marker genes.

(5) Among the Wnt signaling components, authors selected Tcf7l2 as a target for further study, as it contains multiple m6A sites across 5'UTR, CDS and 3'UTR regions. It has been implied that YTHDF1 recognizes the m6As on 3'UTR and facilitate the recruitment of translation initiation factors, promoting the translation of its target mRNAs (Cell. 2015 Jun 4;161(6):1388-99). To further confirm that 'Wnt signaling regulates the translation of TCF7L2 in an m6A-dependent manner', it would be nice to see whether YTHDF1 could promote the translation of Tcf7l2 through m6a methylation in 3'UTR. Further, to clarify the importance of YTHDF1-m6A in the regulation of Tcf7l2 expression in Wnt signal transduction pathway, the protein levels of TCF7L2 could be detected in control and Ythdf1-depleted cells in the presence of active Wnt. These pieces of evidence would make the work more compelling, although not absolutely essential.
Response: Following the referee's suggestion, we further investigated the regulation of TCF7L2 by YTHDF1 through m6A in 3'UTR using luciferase assay. Indeed, erasing m6A by silencing METTL3 decreased luciferase activity, indicating the involvement of m6A in TCF7L2 3'UTR translation (Figure EV5C). Knockdown of YTHDF1 decreased luciferase activity, indicating that YTHDF1 mediates the translation of TCF7L2 3'UTR (Figure EV5D). We have incorporated the data into the revised manuscript.

2nd Editorial Decision 18 December 2019

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, all referees now support the publication of your study in EMBO reports.

Before we can proceed with formal acceptance I have these final editorial requests:

- Please add the single figure shown presently in the Appendix to one of the EV figures. I do not think we need a separate Appendix file for this.

- Thanks you for providing the source data for the blots in Fig. EV1. But, as they are significantly cropped, could you provide the source data for all the Western Blot images (main figures and EV figures)? The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data (scans of entire gels or blots) together with the final revised manuscript. Please include size markers for the scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

- Please remove the writing from the scale bars in Figures 4D and EV3. Please define the length of the scale bars only in the respective figure legends. In Fig. EV3E (lower panel) please move up the scale bar bit, as it currently touches the frame of the image.

- In the author contributions, an author TL is mentioned. But no such author is listed on the title page or in our system. Please check. Moreover, authors Jie Xiang, Zhanghui Chen, Chengping Xu and Xiangwei Gao are not mentioned in the author contributions. Please add this information.

- For the heatmap in Fig. 5E, please define the values shown near the heatmap/color range (fold change?) in the respective legend.

- Please remove the titles of the EV tables from the main manuscript text.

- Please add the titles of the EV legends and their legends on the first TAB of the respective excel

files, and upload these afresh for the final revised version. Finally, remove the readme/text files with this information from the submission.

- Please make sure that when uploading your final revised manuscript that the full and same grant information is entered into the system that is mentioned in the manuscript text.

I look forward to seeing the final revised version of your manuscript when it is ready. Please let me know if you have questions regarding the revision.

REFEREE REPORTS

Referee #1:

I am happy that the authors have addressd al my concerns and look forward to seeing this manuscript published in EMBO Reports

---
Referee #2:

Authors have clearly replied to my concerns, for me the paper is now suitable for publication

---
Referee #3:

In this revised manuscript, the authors have thoroughly addressed all of my concerns. Given the complex role of mRNA modification in cancer biology, the reported role for YTHDF1 in Wnt signaling is of significance. The manuscript is now suitable for EMBO Report.

2nd Revision - authors' response 23 December 2019

The authors performed all minor editorial changes.

3rd Editorial Decision 10 January 2020

Thank you for the submission of your revised manuscript to our offices. I have one final request that needs to be addressed before I can proceed with final acceptance.

We noticed that the tables EV1, EV2 and EV3 are too large to be displayed online. These items need to be datasets (data files that will be linked to the article). Please upload these three files as Dataset files, named Dataset EV1, Dataset EV2 and Dataset EV3. Please add the legends for these datasets (describing what they contain) as a new TAB to the respective excel files (as first TAB). Please adjust the naming of the remaining EV tables (these are then Tables EV1, EV2 and EV3), and finally please change/update the callouts for all these EV items in the manuscript text.

3rd Revision - authors' response 11 January 2020

The authors performed all minor editorial changes.

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: Xiangwei Gao
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-49229

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?	The sample size was chosen according to the published papers. Page 14
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Yes. Page 14
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA.
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.	Yes. Page 14
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	Yes. Page 14
5. For every figure, are statistical tests justified as appropriate?	Yes. Page 19, 27-33
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Page 19
Is there an estimate of variation within each group of data?	Yes. Page 19

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

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

http://datadryad.org

http://figshare.com

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

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

http://biomodels.net/

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

Is the variance similar between the groups that are being statistically compared?	Yes. Page 19

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).	Yes. Table EV5
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Yes. Page 14
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Yes. Page 14
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. Page 14

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Yes. Page 16
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.	Yes. Page 16
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	Yes. Page 20
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.