EMBO reports

tRIP-seq reveals repression of premature polyadenylation by co-transcriptional FUS-U1 snRNP assembly

Akio Masuda, Toshihiko Kawachi, Jun-ichi Takeda, Bisei Ohkawara, Mikako Ito, Kinji Ohno

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision from The EMBO Journal 30 July 2019

Thank you for submitting your manuscript for consideration by The EMBO Journal. We have now received three referee reports on your study, which are included below for your information. Based on these reports, we unfortunately had to conclude that we cannot offer publication at The EMBO Journal at the current stage.

As you will see, all referees acknowledge an interest in the method you develop to detect RNA-protein interactions with increased sensitivity. At the same time, as indicated especially by referee 3, it is apparent that the newly-developed approach has at present not yet been utilized to derive major new biological insights or datasets. Moreover, we feel that the suitability of your experimental set-up for proving that ternary complexes containing proteins in immediate vicinity to one another exist, remains to be decisively demonstrated, given several well-taken technical concerns with the potential to confound key conclusions, as explained by referee 3. In light of these overriding concerns and since it is uncertain whether they can be easily addressed, I am afraid that we find this study currently too preliminary to justify concrete further consideration at The EMBO Journal.

That said, should future work allow you to decisively validate your approach by addressing the methodological concerns, as well as enabling you to derive a valuable new dataset(s) from its application, we would remain open to looking at the study once more as a potential Resource Article. Given the major changes and amendments that would be necessary in such a case, this would however have to be in the form of a new submission, whose novelty at the time of resubmission would then be taken into account as well.

I regret that I cannot be more positive at this stage, but hope that you will nonetheless find the comments of our reviewers helpful. Thank you again for giving us the opportunity to consider your manuscript.

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REFEREE REPORTS

Referee #1:

The manuscript of Masuda et al presents extremely interesting new methodology that is validated by an analysis of FUS function in PolyA definition. I am impressed by the data and the results obtained. I have a few observations as follows:

1- The figures are complex and at points difficult to read, this is a consequence of the enormous amount of data presented, if possible it would be useful to improve the definition of the figures
2- Fig 3C: In the FUS panel there is a signal in the control lane, can the authors comment on it?
3- Fig 3F: The activated by FUS peak in Fig 3F is weaker than the repressed by FUS, this is unexpected because in Page 10 the authors state that there are 26764 APA sites repressed by FUS and 46581 activated by FUS. However the respective peaks in Fig 3F do not represent this difference, on the contrary the FUS activated peak seems to be marginal while the FUS repressed peak is sharp and clear. As the IP was done with anti FUS antibodies I would have expected the inverse if all the binding sites mapped were active. If my reading is correct, the authors should add further discussion on this, it contrasts with the almost quantitative correlation in Fig 4 of FUS binding in the splice sites and with their previous (2015) observations in the FUS silencing experiments.

Referee #2:

Masuda and co-workers have developed a simplified and more sensitive method than eCLIP and HITS-CLIP for the analysis of RNA binding proteins. This method referred to as tRIP is based on the digestion by terminator 5' phosphate dependent exonuclease (TEX) of the RNA in UV-crosslinked RNA-protein complexes down to the position of the antibody bound to the targeted RBP. The data show that this technique shows similar specificity as ClIP analyses but requires much less cells and thus appears to be more sensitive than ClIP.
The authors have employed this technique to analyze the role of ternary protein-protein-RNA complexes and of FUS (a protein that is known to bind upstream of APA sites), RNA polymerase II and U1snRNP in the regulation of alternative polyadenylation (APA). The authors show convincingly by tRIP that U1snRNP and FUS bind upstream of APA in the nascent RNA in the chromatin fraction of cells and show that CPSF binding upstream of APA sites that are suppressed by FUS is increased under conditions of FUS depletion. In experiments depleting either U1snRNP or FUS, the authors show that either depletion is sufficient to lose APA suppression thus indicating that both factors are required and co-operate to regulate APA on nascent RNAs. These data thus indicate by employing a new and sensitive method to analyze RNA-protein interactions an interesting and previously unknown activity of FUS and U1snRNP and likely CPSF160 to regulate APA while the RNA is still engaged with RNAPII in the chromatin.
Finally, the authors link deregulated to APA ion FUS-mutated patients with ALS. This medical perspective of the paper would be substantially strengthened by directly showing evidence of deregulated APA in cells of ALS patients.
In sum, this paper reports an interesting new method for the analysis of complex and low abundance RNA interactomes and reports new and well controlled data on the mechanism controlling alternative polyadenylation.

Referee #3:

Masuda et al, "Identification of U1 snRNP-FUS-RNA interactome co-transcriptionally assembled on RNA polymerase II", aims to identify interactomes of FUS and U1 snRNP on nascent RNA Pol II. They describe a UV crosslinking-immunuprecipitation (CLIP) method, called targeted RNA immunoprecipitation (tRIP), designed to capture and map the RNA binding sites of the IP target and proteins in close proximity to it. To do that, they digest the RNA from the target IP CLIP and perform a second IPs on the released material with antibodies to a protein presumed to be in close proximity to the target. This is a nice idea, which is applied here to FUS and U1 snRNP protein (U1C) with a focus on the potential role of both entities in alternative polyadenylation regulation.

The topic is of general interest from several angles, including the mechanism of U1 snRNP suppression of polyadenylation signals, the potential link to ALS (which can be caused by mutations in FUS), and the potential general utility of the method.
However, in my view, there are major problems with this manuscript, related to the degree of novelty on the biology and the methodology.
1) The method is only useful if the RNA fragment size for the first IP is short enough to ensure that proteins released by the on-beads RNase are bound to the RNA in immediate proximity to the target protein (in fact, the authors use the term, ternary complex, which is more of a claim). This does not seem to be the case. The Methods section states they were ~500-1,000. It is almost certain that numerous other proteins bind to the RNA within that distance. Is there a reason to assume they are less or more relevant than the 2-3 protein pairs probed in this study.
2) The title and text claim discovery of interactomes, generally meant to include mass spectrometry, which was not done here. (It was in fact done and published by others for the same proteins). Figure 3 shows westerns that include very few selected proteins that already known to be interactors. Multiple controls need to be performed and shown, including abundant RNA binding proteins, histones, etc.
3) The manuscript is almost entirely a description of the methodology. The title does not reflect the contents and conclusions of the manuscript. There is very little new biology that I could find.

Additional comments
1) The paper is very difficult to go through. It meanders from RNA modifications to TEX nuclease etc, drowning in detail
2) A window of 2,000 nt was used around APA or AS sites for calculating tRIP read density. This bin size is quite large, and one worry could be that the authors are losing potential binding peaks because of the presence of other APA sites nearby or protein binding locations around splice sites in the upstream exon. It may be beneficial to analyze the average/median distance between two APA/AS sites or between an APA site and the upstream 3'ss in the terminal exon. Then this distance could be used as a bin for computing tRIP coverage.
3) The reads distribution profiles (ex. Figure 3E and 3F) should be of higher resolution, as it is currently very difficult to see the finer essential details.
4) Figure 2A and 2B are not well aligned. Why there is no dilution of cell amount for RBPOX2 tRIP? What is the cell amount for the one shown here?
5) Figure 2G, do the authors have any comments why the distribution form this U shape along genes, with the reads aggregated on the TSS and TTS? It looks like the background contAb-tRIP level is close to the FUS-tRIP (comparing the normalized RPMs), how do the authors explain this?

Authors' appeal at The EMBO Journal 21 November 2019

We greatly appreciate the comments from the reviewers, which have helped to improve our manuscripts. Hereafter, we will provide point-by-point responses, citing the reviewers' comments.

<Reviewer 1>
Comments to Author
The manuscript of Masuda et al presents extremely interesting new methodology that is validated by an analysis of FUS function in PolyA definition. I am impressed by the data and the results obtained. I have a few observations as follows:

Comment 1-1. The figures are complex and at points difficult to read, this is a consequence of the enormous amount of data presented, if possible it would be useful to improve the definition of the figures.

Answer1-1. We apologize for presenting complicated figures. According to the reviewer's suggestion, we enlarged Figures 1FGH, 2DE, 3ABCD, and 4CDE. Additionally, we have made a corresponding table of tRIP-seqs and Figures (Supplementary Table S4).

Comment 1-2. Fig. 3C (currently Fig. 2B): In the FUS panel there is a signal in the control lane, can the authors comment on it?

Answer 1-2. The signal in the control lane was a smear from the band of IgG heavy chain. We replaced the picture of the FUS panel with clearer one (Fig. 2B).

Comment 1-3. The activated by FUS peak in Fig. 3F (currently Fig. 2E) is weaker than the repressed by FUS, this is unexpected because in Page 10 the authors state that there are 26764 APA sites repressed by FUS and 46581 activated by FUS. However the respective peaks in Fig 3F do not represent this difference, on the contrary the FUS activated peak seems to be marginal while the FUS repressed peak is sharp and clear. As the IP was done with anti FUS antibodies I would have expected the inverse if all the binding sites mapped were active. If my reading is correct, the authors should add further discussion on this, it contrasts with the almost quantitative correlation in Fig 4 of FUS binding in the splice sites and with their previous (2015) observations in the FUS silencing experiments.

Answer 1-3.
We thank the reviewer for these insightful comments. The ordinate in Fig. 2E indicates "normalized average tRIP read density". In the calculation of "normalized average tRIP read density", the average of tRIP read density was calculated for each nucleotide position in all sites considered. Thus, "normalized average tRIP-read density" shows the averaged distribution of tRIP-seq reads around an APA site, irrespective the number of analyzed sites. As the reviewer sharply pointed out, RNAPII-FUS-tRIP reads were enriched and diminished upstream and downstream to FUS-repressed APA sites, respectively, which was not evident in Chr-FUS-tRIP. The upstream FUS-binding is required to suppress APA, as demonstrated in the present study. The diminished downstream FUS-binding may represent the formation of FUS-RNA interactions after the dissociation of RNA from RNAPII. We added discussions about these issues in the results section, as recommended.

<Reviewer 2>
Comments to Author
In sum, this paper reports an interesting new method for the analysis of complex and low abundance RNA interactomes and reports new and well controlled data on the mechanism controlling alternative polyadenylation.
The authors link deregulated to APA on FUS-mutated patients with ALS. This medical perspective of the paper would be substantially strengthened by directly showing evidence of deregulated APA in cells of ALS patients.

<Author reply>
Thank you for the insightful comments that improved our manuscript. We established a N2A cell line carrying the ALS-causative NLS-truncation mutation of FUS, R495X, which reduces the interaction between FUS and U1 snRNP. We performed polyA-seq analysis of these cells and found that the mutant FUS aberrantly activated the APA sites, which were normally repressed by FUS-U1 snRNP complex. We also performed the analysis of APA using the previously reported RNA-seq of human motor neurons derived from iPS cells carrying the FUS P525L mutation. The analysis showed the similar misregulation of APA in these cells. We showed these results in a new Figure 5.

<Reviewer 3>
Comments to Author
The topic is of general interest from several angles, including the mechanism of U1 snRNP suppression of polyadenylation signals, the potential link to ALS (which can be caused by mutations in FUS), and the potential general utility of the method.
However, in my view, there are major problems with this manuscript, related to the degree of novelty on the biology and the methodology.

Major comments
Comment 3-1. The method is only useful if the RNA fragment size for the first IP is short enough to ensure that proteins released by the on-beads RNase are bound to the RNA in immediate proximity to the target protein (in fact, the authors use the term, ternary complex, which is more of a claim). This does not seem to be the case. The Methods section states they were ~500-1,000. It is

almost certain that numerous other proteins bind to the RNA within that distance. Is there a reason to assume they are less or more relevant than the 2-3 protein pairs probed in this study?

Answer 3-1. We thank the reviewer for raising these points, and apologize for using the confusing term. As the reviewer sharply pointed out, more than two proteins may be bound to an RNA fragment in the first IP. We used the term "ternary complex" to simplify the model of protein-RNA complexes in the immunoprecipitated machinery. We have quit the use of "ternary" in the current manuscript to prevent misunderstanding of the method. In addition, we remade Figures 2AC, which show the analytical methods of protein-RNA complexes in the RNAPII machinery, to clearly indicate the existence of multiple proteins on an RNA in the first IP. These figures also may help readers to understand that the method is useful even if other proteins are located between the 1st-immunprecipitated protein and a target protein on RNA.

Comment 3-2. The title and text claim discovery of interactomes, generally meant to include mass spectrometry, which was not done here. Figure 3 (currently, Figure 2B) shows westerns that include very few selected proteins that already known to be interactors. Multiple controls need to be performed and shown, including abundant RNA binding proteins, histones, etc.

Answer 3-2. We agree with the reviewer's comment that the term "interactome" has been widely used for mass spectrometry analysis. We now used the term "interactions" instead of "interactome". In Figure 2B, we added the analysis of Histone H3 and 3' end processing factors, including CPSF160, CstF64, and CFIm25, as suggested. Our analysis showed the recruitments of multiple RBPs on nascent RNA engaged to RNAPII.

Comment 3-3. The manuscript is almost entirely a description of the methodology. There is very little new biology that I could find.

Answer 3-3. We thank the reviewer for this comment. To clarify biological roles of the identified FUS-U1 snRNP interaction, we newly investigated the involvements of ALS-causative FUS mutations in the regulation of APA. We obtained a N2A cell line carrying the NLS-truncation mutation of FUS, R495X, which reduces the interaction between FUS and U1 snRNP. We performed polyA-seq analysis of these cells and found that the mutant FUS aberrantly activated the APA sites, which were normally repressed by FUS-U1 snRNP complex. Similar misregulation of APA was also observed in RNA-seq of iPS cells carrying the FUS P525L mutation, which also disrupts FUS-U1 snRNP interaction. In addition, gene ontology analysis showed that these misregulated APA sites were enriched in genes involved in neuronal functions. These observations suggest a role of the identified FUS-U1 snRNP interaction in the pathogenesis of ALS. We showed these results in a new Figure 5 and Table S2.

Minor comments

Comment 3-4. The paper is very difficult to go through. It meanders from RNA modifications to TEX nuclease etc, drowning in detail.

Answer 3-4.

We apologize for the complicated manuscript. We have simplified the descriptions about RNA modifications and TEX treatment, and have focused on the analysis of protein-RNA interactions especially in the RNAPII machinery. Additionally, we have made a corresponding table of tRIP-seqs and Figures (Supplementary Table S4).

Comment 3-5. A window of 2,000 nt was used around APA or AS sites for calculating tRIP-seq read density. This bin size is quite large, and one worry could be that the authors are losing potential binding peaks because of the presence of other APA sites nearby or protein binding locations around splice sites in the upstream exon. It may be beneficial to analyze the average/median distance between two APA/AS sites or between an APA site and the upstream 3'ss in the terminal exon. Then this distance could be used as a bin for computing tRIP-seq coverage.

Answer 3-5.

Thank you for the comments. The average distance between adjacent APA sites is 56,438 nt (median, 5,065 nt), and that between adjacent AS sites is 35,677 nt (median, 1961 nt). Thus,

APA/AS sites are not frequently present in a window of 2,000 nt (±1,000 nt from an analyzed site). We added descriptions about the distance between the adjacent sites in the methods section.

Comment 3-6. The reads distribution profiles [ex. Figures 3EF (currently, Figures 2DE)] should be of higher resolution, as it is currently very difficult to see the finer essential details.

Answer 3-6.
We apologize for presenting complicated panels. We enlarged Figures 1FGH, 2DE, 3ABCD, and 4CDE.

Comment 3-7. Figure 2A and 2B are not well aligned. Why there is no dilution of cell amount for RBPOX2 tRIP? What is the cell amount for the one shown here?

Answer 3-7.
tRIP-seq of RBFOX2 was performed using 2×10^7 cells, which is now shown in Figure 2B. We used the same cell line (HEK293T), the same cell number (2×10^7 cells) and the same antibody, as were used in the eCLIP of RBFOX2, for comparison. Although we successfully performed tRIP-seq of RBFOX2 using 5×10^5 cells, we failed to make tRIP-seq libraries using less cells such as 1×10^5 cells and 5×10^3 cells, probably due to the low abundance of RBFOX2-RNA complexes in these cells or due to the inefficiency of the antibody to immunoprecipitate trace amounts of the complexes. Since tRIP-seqs of FUS, PTBP1, and m6A can be performed using much less cells (Figure 1C and Supplementary Figures S1KLM), we did not show the results of RBFOX2-tRIP using 5×10^5 cells.

Comment 3-8. Figure 2G (currently Figure 1H), do the authors have any comments why the distribution form this U shape along genes, with the reads aggregated on the TSS and TTS? It looks like the background contAb-tRIP level is close to the FUS-tRIP (comparing the normalized RPMs), how do the authors explain this?

Answer 3-8.
Thank you for the comment. Accumulations of FUS around TSS and TTS have been shown in the previous reports. We added the description of the distribution of FUS-tRIP reads in the results section, referring these reports. The comparable level of contAb-tRIP was due to the small number of total mapped reads, since RPM is the ratio of the number of reads at each position to total mapped reads. As the NGS-plot analysis of contAb-tRIP was confusing, we have removed the figure in the current manuscript.

2nd Editorial Decision from the EMBO Journal 3 December 2019

Thank you for submitting a revised version of your manuscript EMBOJ-2019-102764, which had been rejected post-review earlier. I have now looked at the revised manuscript, your point-by-point response and discussed this as well as the initial decision with other members of the editorial team. I regret to inform you that we have come to the conclusion that we cannot offer to consider the study further for publication at The EMBO Journal.

We appreciate that you have performed additional experiments and added to the discussion to address the referees' initial comments. In addition to the more technical issues regarding the new methodology, one of the major issues referee #3 had pointed out in the initial comments was the identification of a limited set of interactors (3-2). We had referred to this in our initial decision letter and had noted that we would be open to reconsider the study as a Resource Article reporting and validating a novel method, if you were able to address the technical issues and derive a new dataset valuable to the field as such. We realize that you have addressed referee #3's concern by now also including Histone H3 and 3' end processing factors. However, to be considered as a Resource Article, the dataset would need to be more extensive and derived from a more a global and unbiased approach.

We recognize that you have also added an analysis of two ALS-associated FUS mutations, which reduce its association with U1snRNP, and find that the suppression of APA is impaired, particularly in genes involved in neuronal functions. This leads you to conclude that mutations that disrupt the

FUS U1 snRNP interaction lead to an activation of APA sites. These findings further support your previous conclusion that binding of both U1 snRNP and FUS is needed to suppress APA sites. However, we find that for an EMBO Journal Research Article, a more in depth mechanistic analysis of the proposed model, as well as a demonstration of its physiological role, would be required. Thus, overall we unfortunately have concluded that the study is currently not suited for further consideration at EMBO Journal.

This decision however only reflects our assessment of the suitability for this particular journal, and we appreciate the quality of your study and recognize that the tRIPseq approach and the findings will be of interest to other researchers in the field. During your submission, you indicated that we should not discuss your study with editors at other EMBO Press journals. However, we think your study in its current form may well be a candidate for our sister journal EMBO reports and would like to encourage you to reconsider this decision. If you would be interested in transferring your manuscript with the previous referee reports to EMBO reports, please either contact me so that I can pre-discuss your work with the responsible editor at EMBO reports, or directly contact EMBO reports.

Thank you again for the opportunity to consider your study. I am sorry that we could not be more positive on this occasion.

1st Editorial Decision from EMBO reports 11 February 2020

Thank you for the submission of your revised manuscript. We have now received the enclosed comments from the referees and I am happy to say that both support its publication now.

However, a few minor editorial changes are required before we can proceed with the official acceptance of your manuscript.

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have any questions or comments.

REFEREE REPORTS

Referee #1:

The authors have clarified all the queries of this referee so I consider the manuscript is suitable for publication in EMBO Reports

Referee #2:

The suggested changes during the first round of Review have been implemented and the paper now appears to be suitable for publication

Referee #3:

The authors have revised and clarified the manuscript. The figures have been corrected and simplified, and probed additional proteins from the IPs, as suggested. The methodology is described more clearly. Concerns about the data analysis have been addressed.

2nd Revision - authors' response 19 February 2020

Authors made the requested 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: Akio Masuda
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-49890-T

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.

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?	Preliminary data originated in the laboratory and results from parallel experiments were used to estimate sample size.
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?	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.	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. Every statistical analysis performed on the data is explained in the corresponding figure legend.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We used JMP pro 14.2.0 software for reasonable data analysis.

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

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).	All antibodies are listed in the Appendix Table S5.
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.	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	All tRIP-seq and polyA-seq data generated for this study have been deposited in the DDBJ Sequence Read Archive (https://www.ddbj.nig.ac.jp/dra/index-e.html). The accession numbers are listed in Appendix Table S4.
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).	Raw data will be available for graphs and statistics upon request, and this is clearly stated in our manuscript for readers.
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