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PPR polyadenylation factor defines mitochondrial mRNA identity and stability in trypanosomes

Liye Zhang, Francois M. Sement, Takuma Suematsu, Tian Yu, Stefano Monti, Lan Huang, Ruslan Aphasizhev & Inna Aphasizheva

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Editorial Decision:	29 March 2017
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Editor: Anne Nielsen

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 29 March 2017

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below.

As you will see from the reports, our referees all express interest in the findings reported in your manuscript, although they also list a number of concerns regarding controls and data interpretation that you will have to address before they can support publication in The EMBO Journal. You will see that referees #2 and #3 mainly ask for text clarifications and further discussion while ref #1 finds that additional experimental data is needed to support direct and selective binding of KPAF3 to mRNAs. In addition, the referee suggests a number of controls that should be straightforward to include, and which would in our view further strengthen the conclusiveness of your study.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the

conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

In the manuscript entitled "PPR polyadenylation factor defines mitochondrial mRNA identity and stability in trypanosomes" the authors identify the novel PPR factor KPAF3 as a necessary component in the poly adenylation of Trypanosome mitochondrial mRNAs, which has a direct consequence on mRNA stability. Overall, the data is of high quality but that being said there appears to be a rather selective interpretation of the data in specific experiments that cast doubt on the robustness of the conclusions and as a result the proposed mechanism for KPAF3 function. These points should be addressed by the authors in more detail to delineate the function of KPAF3 on mitochondrial RNAs in Trypanosomes.

Major points.
1. The authors claim the function of KPAF3 is specific to mitochondrial mRNAs, but the data in Figure 3 disputes this interpretation. In particular, Figure 3D strongly indicates the abundance of 12S and 9S are affected by the KPAF3 RNAi, and to a lesser extent a similar observation is seen for gMurf2(II) and gA6(14). Although, the effect is subtle for guides RNAs, the difference does not appear to due to loading differences so a lower exposure and some quantification would be revealing. Since a central claim of the manuscript is the specificity of KPAF3 for mRNA and their polyadenylation, the authors need to address why there are changes in the mitochondrial rRNA and guide RNAs with KPAF3 RNAi.

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The authors have extended their earlier studies of mitochondrial mRNA biogenesis and turnover in trypanosomes to characterize the role of the KPAF3 RNA-binding protein. They first identify a role for KPAF3 in mRNA stability by performing a targeted RNAi screen of pentatricopeptide repeat-containing RNA binding proteins, and proceed to characterize protein interactors of KPAF3 by tandem affinity purification and mass spectrometry. Inducible RNAi knockdown indicates an essential role of KPAF3 in vivo, and the data presented indicate that this involves regulation of mRNA stability in a manner that depends on the extent of prior mRNA editing. Interestingly, KPAF3 is shown to stabilize mRNA targets in a manner that is independent of their polyadenylation state, but also to promote mRNA polyadenylation by KPAP1. Further data indicate that KPAF3 inhibits RNA degradation by the mitochondrial 3′ processome in vitro, and characterization of RNAs bound by KPAF3 suggests that KPAF3 binding serves to distinguish functional mitochondrial mRNAs from those destined for degradation. UV crosslinking is used to define a G-rich octamer as the consensus KPAF3 binding site, and the functionality of this binding site is demonstrated in an artificial substrate. This consensus site is more frequent in pre-edited RNAs than in fully edited mRNAs, providing the basis of a model where bound KPAF3, which stabilizes pre-edited RNAs, is displaced as a result of RNA editing, which tends to destroy KPAF3 binding sites. The data, though abundant, are of a very high overall quality, and the manuscript is well written.
This is a complex story, but the inclusion of the explanatory Figure 9E helps to make the study accessible to readers with interests in RNA metabolism outside the trypanosome field.

The authors may wish to address the following minor points:

1. (Page 7): "Repression of Tb927.9.12770 produced the expected phenotype" should be re-phrased; as I understand it, the phenotype of Tb927.9.12770 RNAi was not previously known.

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2. (Legend to Fig. 1): 'non-denylated' should read 'non-adenylated'.

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Although the nature of the editosome and the basic editing mechanism has been elucidated still many questions remain about the decision making for translation of edited mRNAs. A standing conundrum is as to how only edited mRNAs make it to the translating ribosome while pre-edited mRNAs do not. Should the latter occur it could produce a backlog of defective proteins that may be detrimental to cells.
The present manuscript identifies the PPR protein KPAF3 as an essential player in this decision-making. As clearly shown it is this factor that determines the nature of the tail that mRNAs get, which is crucial to differentiate the various transcripts made in the kinetoplast. The work is of outstanding technical quality and the experiments are thorough and insightful. The manuscript is also very well written and explains a complex subject with clarity of detail. I just have two points that do not require experimentation but that should be address in the discussion. The authors have raised a parallel with the plant systems and the abundance of PPR protein in trypanosomes with ~20 associating with polyadenylation. In addition, some of the edited RNAs (fig 2.C, Cox3 and Cox2) do not seem to change in editing efficiency when KPAF3 is down regulated. Is it possible other PPR proteins are then utilized for such mRNAs and that like in plants there are different PPRs for different messengers? At least the authors should speculate on why those do not appear to change. Otherwise a great story worthy of the EMBO J.

1st Revision - authors' response 10 April 2017

General comments

We'd like to thank the Referees for their diligent efforts in analyzing a rather substantial volume of data, and providing fair and scrupulous critiques. These were most instrumental in improving the manuscript. Referee 1 pointed out a "*rather selective interpretation of the data in specific experiments*," which we had tried to avoid while balancing the level of details with accessibility for a broader audience. Although *Trypanosoma brucei* is classic organism for studies of mitochondrial RNA biology, some of the RNA processing mechanisms are arguably unconventional, and their description often requires substantial background information. With space limitations in mind, we were compelled to deliver the most important points in greater details, perhaps at the expense of uniformly deep coverage for each of 44 panels in nine figures. In this context, it was helpful to see which items require clarification.

Point-by-point responses

Referee #1:

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Indeed, a mRNA-specific effects of KPAF3 knockdown should have been better articulated and impact on rRNAs and gRNA discussed. It appears that rather substantial (~50%) downregulation of 9S and 12S rRNA in KPAF1/2 RNAi caught the Reviewer's attention in Figure 3D (quantitation provided in Appendix Table S2), whereas the effects of KPAF3 shown in the same panel were modest (upregulation to ~130% at 48 h of RNAi induction, and then return to ~100% by 72 h). Since this study is centered on KPAF3, we felt that elaborating on KPAP1/2 RNAi effects on rRNAs would skew the narrative from the main topic. The KPAP1/2 effects were most likely caused by the lack of translatable mRNAs and inhibited translation in this cell line (Aphasizheva et all, Mol Cell, 2011). We agree, however, that the question of KPAF3 RNAi effect on rRNAs is important because both pre-rRNAs and pre-mRNAs are transcribed from maxicircle DNA. The Northern blotting analysis was repeated

using an agarose-formaldehyde gel instead of PAGE-8M urea to eliminate band compression and allow for better quantitation of long rRNAs. The KPAF3 panel in the Figure 3D was replaced with new gels and quantitation updated in Appendix Table 2, but the results were virtually identical: a minor upregulation by mid-course of RNAi, and then return to baseline. We also keep in mind that qRT-PCR analysis did not reveal major difference in 9S and 12S rRNA relative abundance at 72 h of RNAi induction (Figure 2C). This was also the case in original RNAi screening of candidate PPR proteins (Figure 1A). Overall, we feel that the conclusion of rRNA not being significantly affected by KPAF3 RNAi is now quite solid.

Technical note: all images were acquired before reaching saturation, typically at no more than 50% of dynamic range; scanner data files are available for review. Change of contrast and resizing were the only image manipulations used in this work.

The moderate guide RNA upregulation in KPAF3 RNAi, on the other hand, is authentic (Figure 3E). In previous work on RNA editing substrate binding complex (Aphasizheva et al, MCB, 2014), we have demonstrated guide RNAs upregulation in several genetic backgrounds with inhibited RNA editing. This upregulation appears to be a generic output of any interference with the editing process, e.g., knockdown of an editing enzyme or structural complex component. We further showed that guide RNAs are degraded, rather than recycled, during active editing process. It follows that depletion of pre-edited mRNA in KPAF3 RNAi cell line causes inhibition of editing at later time points (Figure 3A), hence, accumulation of gRNAs. Initially omitted for reasons and space and focus on mRNA, this explanation has been incorporated into the revised manuscript (page 11, last paragraph).

2. The interpretations of the northern data following KPAF3 RNAi is a bit puzzling for this reviewer. In Figure 3A, the data for the KPAP1 RNAi fits with the described function of this protein: no poly adenylation of mRNAs in its absence. In contrast, for the KPAF3 RNAi there is severe reduction in the abundance of the protein by 24 hours and absence by 48 hours (Figure 2A), yet the biggest effect on the various mRNA species is seen at 72 hours. As mentioned in point 1, there is an effect on the rRNA and a considerable difference in the abundance of 12S at 24hours. What accounts for the delay in the decay of the mRNA if there is no KPAF3 present?

These keen observations by the Referee 1 reflect the rationale in our approach of initially analyzing the overall change in mRNA relative abundance by qRT-PCR at single RNAi time point (Fig. 2C), and then performing time-resolved high-resolution Northern blotting. The latter reveals changes in steady-state levels for RNA editing substrate (pre-edited mRNA), intermediate (70% edited mRNA), and product (fully-edited mRNA). Gradual protein depletion by RNAi permits monitoring of "does-response" events and is instrumental in defining the order of events. Along with oligo[dT]/RNase H control and KPAP1 poly(A) polymerase RNAi outcome (loss of A-tail), we could detect initial gradual loss of A-tail in pre-edited mRNA upon KPAF3 repression (24-48 hours), followed by a rapid degradation of the entire mRNA at 72 hours. It is only fitting that major effects on partially- and fully-edited mRNAs were observed starting at 72 hours since the precursor (pre-edited mRNA) had been virtually eliminated at this point. The Referee's argument points out a gap in our knowledge regarding the number of KPAF3 molecules and their RNA targets in the cell, and a minimal KPAF3 levels required to sustain pre-edited mRNAs. Although filling such gap properly

would be beyond the scope of this study, it seems reasonable to assume some interval between protein downregulation, and changes in polyadenylation status and abundance of its RNA targets. Please also see discussion for Major Point # 4.

3. The authors claim that KPAF3 binds mRNAs lacking an A tail and stabilize the message as seen for the KPAP1 RNAi (Figure 3A). A nice control would be a KPAP1 and KPAF3 double RNAi experiment, which should reveal that the stability of RPS12 mRNA lacking an A tail would be lost.

We thought about such experiment, but decided not to proceed. We reasoned that KPAF3 RNAi phenotype (gradual loss of A-tail followed by rapid pre-edited mRNA degradation) would supersede KPAP1 phenotype (rapid loss of A-tail followed by pre-edited mRNA stabilization). In other words, the outcome of dual KPAP1-KPAF3 RNAi would look just like KPAF3 knockdown.

4. There seems to be discrepancies in the data of Figure 4. In Figure 4A, the 0 time points for the Mock at 36 and 48 hrs. appear vastly different, should that be the case? Also, at 36 hours for the KPAF3 RNAi in the 0 time point of the washout there appears to be an accumulation of no A-tail, a finding not seen in Figure 3, please explain?

These are correct observations that indicate a need for more explicit explanation, now added to the text (Page 12 and legend to Figure 4A). The rationale for selecting these RNAi time points, as stated, was to look at "The time points when pre-edited RPS12 mRNA is already deadenylated, but not yet degraded (36 hours), and when the bulk of mRNA is degraded, but some mRNA remains (48 hours) were selected as starting points." Because pre-edited mRNA is already downregulated at 48 hours (compare "RNAi" panels in Fig 4A at 36 and 48 h, we had to increase contrast in the entire "48 h" panel to make the pre-edited mRNA at zero ActD visible. Naturally, the mock induced panel look overblown. This was mentioned in the Figure 4 legend: "Contrast was increased in the right panel to reflect RNA loss at 48 hours of KPAF3 RNAi." We must stress that quantitation of relative abundance was not affected since zero ActD sample was assumed to be 100% in all cases. These experiments were only possible because the effects on RNA lag slightly behind protein depletion (see response to Major point 2). Hence, we were able to capture decay kinetics when RNA is already non-adenylated, but not yet degraded (36 hr), and non-adenylated and already largely degraded (48 h). The experiment is admittedly subtle, but it illuminates a key point: KPAF3 plays an essential role in mRNA polyadenylation, but its stabilizing function does not depend on the A-tail's presence. These findings were latter corroborated by *in vitro* and *in vivo* experiments (Figures 5-8). Finally, minor accumulation of "no A-tail" form at 36 hours for the KPAF3 RNAi in the zero timepoint is entirely consistent with Figure 3A: loss of the A-tail, whether continuous throughout KPAP1 RNAi or short-lived in KPAF3 RNA, causes minor pre-edited mRNA accumulation. In this work, we made no claims of understand why pre-edited mRNA slightly accumulates in KPAP1 RNAi. Again, we appreciate Reviewers' attention to minute details, but fear that diving into more extensive discussion will have most readers lost.

5. The data from Figure 5 appear to this reviewer to suggest that there is a nucleotide preference for KPAF3 at the 3'end, which is the opposite conclusion made in the manuscript.

We believe that the Referee refers to Figure 5D, in which we tested whether polyadenylation factor KPAF3 alters KPAP1's preferences for the nucleotides at the 3′ end of RNA substrate. This experiment was performed as foundation for subsequent figures showing that *in vivo* KPAP1 acts on 3′ termini produced by MPsome-catalyzed 3′-5′ pre-mRNA degradation, which often leaves behind short (1-5 residues) tracts of uridines. KPAP1 alone apparently does not discriminate RNAs terminating with 6 As and 6 Us (lanes in Figure 5 D marked as "KPAP1" next to "[6A]RNA" and "6[U]RNA." Hence, the main question was whether KPAF3, while stimulating KPAP1 processivity, also alters RNA substrate preference. The reaction appears to be less efficient on [6C]RNA and inhibited on [6G]RNA, most likely due to G-quadruplex formation. However, we contend that biologically relevant substrates are [6A]RNA and [6U]RNA, which were utilized equally efficient.

Also, what is the discrepancy between 5F and 5D for UTP and CTP?

Figure 5F addresses a somewhat similar question to that described above, but now looking at NTP selectivity of KPAP1 poly(A) polymerase in the presence of KPAF3. Again, there is more to the question that could be discussed in detail, but we have happy to do it here. TUTases and non-canonical poly(A) polymerases, such as KPAP1, often display mixed NTP specificities. For example, Cid1 protein from *S. pombe* was initially identified as poly(A) polymerase, but later demonstrated to have preference for UTP *in vivo*. This phenomenon was attributed to influence of interacting proteins (series of studies from Chris Norbury). Hence, it was important to see whether KPAP1's NTP specificity changes upon stimulation by KPAF3. In Figure 5F we reproduced our previous results on KPAP1's ATP preference and showed that KPAF3 simulates UTP and CTP utilization to much lesser extent than ATP polymerization. Hence, the conclusion was reached that although KPAP1 is simulated by KPAF3, the enzymes' NTP selectivity is not affected by PPR factor. We feel this is important.

We are not quite sure what does the Referee mean by "UTP and CTP" in Figure 5D; the entire experiment was conducted with ATP, only RNA substrates changed. This is stated in figure legend.

Minor point

1. In Figure 5E, right panel, there appears to be a mistake in the labelling as the KPAP1 lane would be expected to have the addition of KPAP3 to account for the effect seen and difference with the left panel (the absence of KPAP3).

Yes, of course. In the right panel, the bracket indicating KPAF3 addition to the reaction should have been extended to include "KPAP1" lane. Corrected. We could not be more thankful for Referee's thoroughness.

Referee #2:

The authors have extended their earlier studies of mitochondrial mRNA biogenesis and turnover in trypanosomes to characterize the role of the KPAF3 RNA-binding protein. They first identify a role for KPAF3 in mRNA stability by performing a targeted RNAi screen of pentatricopeptide repeat-containing RNA binding proteins, and proceed to characterize protein interactors of KPAF3 by tandem affinity purification and mass spectrometry. Inducible RNAi knockdown indicates an essential role of KPAF3 in vivo, and the data presented indicate that this involves regulation of mRNA stability in a manner that depends on the extent of prior mRNA editing. Interestingly, KPAF3 is shown to stabilize mRNA targets in a manner that is independent of their polyadenylation state, but also to promote mRNA polyadenylation by KPAP1. Further data indicate that KPAF3 inhibits RNA degradation by the mitochondrial 3′ processome in vitro, and characterization of RNAs bound by KPAF3 suggests that KPAF3 binding serves to distinguish functional mitochondrial mRNAs from those destined for degradation. UV crosslinking is used to define a G-rich octamer as the consensus KPAF3 binding site, and the functionality of this binding site is demonstrated in an artificial substrate. This consensus site is more frequent in pre-edited RNAs than in fully edited mRNAs, providing the basis of a model where bound KPAF3, which stabilizes pre-edited RNAs, is displaced as a result of RNA editing, which tends to destroy KPAF3 binding sites. The data, though abundant, are of a very high overall quality, and the manuscript is well written.
This is a complex story, but the inclusion of the explanatory Figure 9E helps to make the study accessible to readers with interests in RNA metabolism outside the trypanosome field.

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1. (Page 7): "Repression of Tb927.9.12770 produced the expected phenotype" should be re-phrased; as I understand it, the phenotype of Tb927.9.12770 RNAi was not previously known.

> Agreed. The sentence in question was replaced with “Repression of Tb927.9.12770 caused degradation of the pre-edited mRNA while leaving 9S and 12S rRNAs unaffected.”

2. (general) The assignment of northern blot signals to 'long A/U tail', '70% edited, short A tail' and so on could perhaps be explained more clearly in the text, in the interests of the general readership.

> Agreed. Additional explanation was added to legend for Figure 3A, including positioning of the hybridization probe used to detect the “70% edited” mRNA.

3. (Legend to Fig. 1): 'non-denylated' should read 'non-adenylated'.

> Corrected.

Referee #3:

Although the nature of the editosome and the basic editing mechanism has been elucidated still many questions remain about the decision making for translation of edited mRNAs. A standing conundrum is as to how only edited mRNAs make it to the translating ribosome while pre-edited mRNAs do not. Should the latter occur it could produce a backlog of defective proteins that may be detrimental to cells.
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The Referee is correct in pointing out that some mitochondrial transcripts are more affected by KPAF3 knockdown than others; this indeed opens a possibility that transcript-specific factors are also involved in mRNA stabilization. Admittedly, we have so far defined functions of only two polyadenylation factors in A/U-tailing (KPAF1/2, Aphasizheva et all, Mol Cell, 2011) and A-tailing/mRNA stabilization (this study). However, our preliminary studies indicate that most of 40+ PPRs in *T. brucei* are essential for parasite viability and we have previously discussed their possible functions in a review article (Aphasizhev and Aphasizheva, RNA Biology, 2013). Clearly, much work lies ahead. A ubiquitous distribution of KPAF3 binding sites in pre-edited and unedited mRNAs would argue for its rather general role, but we were happy to incorporate the Referee's suggestion into Discussion section (page 23, first paragraph).

2nd Editorial Decision 08 May 2017

Thank you for submitting a revised version of your manuscript. It has now been seen by two of the original referees whose comments are shown below (please note that ref #1 did not provide written comments but directly recommended publication). As you will see the referees both find that all criticisms have been sufficiently addressed and recommend the manuscript for publication. However, before we can officially accept the manuscript in The EMBO Journal there are a few editorial issues concerning text and figures that I need you to address.

REFEREE REPORT

Referee #3:

I believe the authors have more than addressed my concerns. This is an extensive and well-controlled piece of work.

2nd Revision - authors' response 10 May 2017

Thank you for your guidance in manuscript preparation. Enclosed please find a revised version and point-by-point responses to editorial comments. My colleagues and I truly appreciate the comprehensive and timely review process.

3rd Editorial Decision 22 May 2017

Thank you for submitting the final revision of your manuscript, I am pleased to inform you that your study has been accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Inna Aphasizheva
Journal Submitted to: The EMBO Journal
Manuscript Number: 96808

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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?	N/A
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
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.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
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.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	yes, statistical tests were applied when needed and described in Method section. Almost all statistical tested performed were based on well established and commonly used algorithms/methods such as MACS, MEME, FIMO.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	Yes, see the error bars and boxplots in the figures.
Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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 were generated in-house and validated in this or previous peer-review studies.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	29-13 Lister 427 Trypanosoma brucei. Wirtz E, Leal S, Ochatt C, Cross GA (1999) A tightly regulated inducible expression system for conditional gene knock-outs and dominant-negative genetics in Trypanosoma brucei. Mol. Biochem. Parasitol 99: 89-101.

* 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.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
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.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
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.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The protein-coding sequence of the KPAF3 gene has been deposited in GenBank under accession code KY645970.
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).	Deep sequencing data have been deposited into the Sequence Read Archive (SRA) under accession code SRP100492.
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).	N/A
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No