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Distinct modes of recruitment of the CCR4-NOT complex by *Drosophila* and vertebrate Nanos

Tobias Raisch, Dipankar Bhandari, Kevin Sabath, Sigrun Helms, Eugene Valkov, Oliver Weichenrieder and Elisa Izaurralde

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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 14 January 2016

Thank you for submitting your manuscript (EMBOJ-2015-93634) 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, all referees express interest in the findings reported in your manuscript and praise the high quality of the data presented. They do have a number of suggestions for improvements/clarifications in the manuscript and would ask you to address these before publication.

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. You will see that the referees are not asking for much new experimental data to be included in the revised manuscript; however I would encourage you to follow their suggestions on improving figure presentation and data description. In addition, several points require a more extended description of the experimental setup to be included in the materials and methods section.

REFEREE REPORTS

Referee #1:

In the manuscript "Distinct modes of recruitment of the CCR4-NOT complex by Drosophila and vertebrate Nanos" submitted to the EMBO Journal, Raisch et al. present a well written and technically sound study on CCR4-NOT Nanos interactions. The structure presented adds to the still few numbers of protein-protein complexes involved in mRNA regulation and furthers our understanding how an RNA binding protein works as a bridge to recruit other factors to assemble a larger complex for, in this case, mRNA degradation. The main finding is how CCR4-NOT gets recruited to the site of Nanos RNA binding by extended structural regions of Nanos in Drosophila. A very surprising feature here is that the regions involved are different from vertebrates (human) and invertebrates (fruit fly), although the NOT regions, where Nanos of both binds to are highly conserved. In general, this study is very important to the field and also has some general interest. I therefore recommend this manuscript to be published in EMBO Journal provided the authors address my following concerns or comments (which I consider requires minor revisions):

1. One of the main points of this study, the surprising difference between Drosophila and humans in their way to recruit CCR4-NOT can be extended a little by adding Nanos alignments between hs and dm Nanos. Especially since the NOT modules are highly conserved, even the NED binding sites on NOT are almost identical. That raises the question if there are other factors in humans, which take up the same sites as NED in Drosophila or the other way around. Can these factors be identified by simple bioinformatics analysis?

2. The authors claim that the deletion of the BoxA motif in hunchback mRNA has "no" effect on Nanos activity, thus they conclude that Nanos binds and represses the hb reporter independently of BRAT. I think, however, that in Figure 2B,C, there is a difference between WT and BoxA deletion. The difference is not large but might raise the question where this difference comes from. Is there some dependence on BRAT for Nanos RNA binding (improving RNA binding) after all, or might deletion of the 5 GUUGU's at NRE1 and NRE2 not be enough? A few weeks ago, the same authors who published the cited paper (Loedige et al., 2014), identifying the GUUGU motif have published another article (Loedige et al., 2015), where the actual and optimal BRAT binding motif has been identified. It seems to be UUGUUG. If you delete GUUGU from NRE1 there isn't something left where BRAT could bind to. However, RNA binding might be retained considerably at NRE2, where GUUGU deletion results in UUUUCG, to which BRAT should still be able to bind.

3. Page 10, last paragraph. Also PAN3 seems to be pulled down, comparing the input between PAN2 and PAN3? Also, it should refer to figures EV1F-L, now it refers only to figures EV1G-L.

4. Page 14, bottom it says:"...adopt an SH3-like fold comprising a five-stranded half-open beta-barrel that mediates dimerization." To me, it looks as if the helical bundle mediates dimerization?

5. The description of structural details and their figures clearly needs revision. Many residues mentioned in the text are not shown in the figures. Especially the explicitly described K737-F152 carbonyl oxygen contact is not visible in the figure and hard to imagine to take place, as the side chain of K737 seems to be rather far away from the F152 carbonyl. Also, from 6E it is hard to see if there is a indeed a contact between F1876 and F130 or L127. The ring-ring interactions seem unusual anyways, maybe due to the low resolution of 3.9 Angström? It is always a pity that the coordinates are not available to the reviewers (for understandable reasons). This makes it hard to judge if the structure has been refined properly. But I trust it has, as experts in the field did it. Nevertheless, might it be worthwhile to look at the interface again?

6. Should Figs 6H-J moved over to Figure 7?

7. Can the authors comment whether they have tried further NED sequences to co-crystallize with the NOT module, which bind in a redundant manner (regions 50-115 and 164-236)? Didn't it work? If it did, why not including here?

8. What about the affinity of the single NED regions to the NOT-module and of both together? It would be interesting to see if there is a cooperative effect or just additive.

9. The addition of more and more acronyms and abbreviations does not make it easier to read.

Wouldn't it be better to call them all NIMs, numbering them and put a species indicator in front? Like hsNIM1 (NIM), dmNIM2 and dmNIM3 (NED - N1BR N3BR)?

10. The authors should very briefly say how the alignments were done and what program was used to display them (at least one citation is therefore missing).

Referee #2:

The CCR4-NOT deadenylase machinery is a multi-subunit protein complex that is recruited to many cellular mRNAs in order to shorten their 3' poly(A) tails and engender their decay. CCR4-NOT is comprised of multiple subunits, including CCR4 and CAF1 deadenylases, which display 3'-5' exonuclease activity. This machinery can interact with a number of gene silencing modules, including miRISC, TTP and Nanos to engender mRNA decay. Vertebrate Nanos was previously reported to interact with the NOT1 subunit of the CCR4-NOT complex via NOT1-interacting motif (NIM); however, this motif is not present in Drosophila Nanos. Thus, how Nanos in several invertebrates interact with the CCR4-NOT complex was unclear.

Here, Raisch and colleagues present a beautiful paper that sheds important light on how Drosophila Nanos interacts with both NOT1 and NOT3 subunits of the CCR4-NOT complex in a manner that is unique to invertebrates. The paper is well-written, and the data are of high quality and novel. This paper should definitely be published in EMBO.

Minor points:

1. On page 11, the authors write 'Deletion of the NOT1 C-terminal region that includes the SHD domain reduced but did not abolish the interaction of Nanos with NOT1 (FIG EV2G, lane 12)'. Considering that GFP-Nanos was not expressed or IP'd as efficiently as in other lanes, I'm not completely convinced that there is any reduction of this mutant in binding NOT1 whatsoever. The authors may want to mention this in their results.

2. The authors should also provide a diagram depicting NOT1 and the deletion mutants that were constructed. It would be of help to the readers who may not know NOT1 structure.

Referee #3:

CCR4-NOT is one of two conserved mRNA deadenylases in eukaryotes. It is a multi-protein complex that is recruited to specific RNAs by a number of RNA binding proteins. Previously, the interaction between vertebrate Nanos (an RNA binding protein) and CCR4-NOT had been characterized. Drosophila Nanos doesn't contain an obvious NOT1 interacting motif (NIM) so it wasn't clear how it would interact with the complex.

Here, the authors define and characterize the interaction between Drosophila Nanos and the CCR4-NOT complex. First, they map the region of Dm Nanos (the NED, Nanos Effector Domain) required for mRNA repression (both by artificial tethering and by direct interaction with hunchback RNA) in Drosophila S2 cells and show that this results in mRNA degradation and translation repression. RNA recruitment is dependent on PUM but not BRAT.

NED binds CCR4-NOT via at least two separate interactions. The authors determine a 3.1 A co-crystal structure of the minimal part of the NED (termed the NBR, NOT-module binding motif) bound to the NOT module (NOT1-NOT2-NOT3). Sequence assignment was confirmed using SeMet as well as by mutation of residues at the interface. The NBR forms two separate interactions with the NOT module, contacting NOT3 and NOT1. Interestingly, Drosophila Nanos NBR interacts with a surface of the NOT module distinct from the NOT1 surface bound by NIM of human Nanos. Thus, although the interacting surfaces are not conserved, the interactions are functionally analogous - this was confirmed using chimeric human-Drosophila proteins.

Overall, this paper reports a number of new and interesting findings regarding RNA recruitment by CCR4-NOT and Nanos. The data are of high quality and the text is clear. This work will be an important contribution to the field.

Specific comments

1. In Figure 3, the authors show experiments from cells where DCP2 is depleted and a catalytically inactive mutant (E361Q) is overexpressed. This approach has been used in other work but it's not clear why both depletion of wild-type DCP2 and overexpression of a catalytically inactive version are required?

2. In Figure 3A, F-luc activity should be shown as well as mRNA levels.

3. How were the mRNA half lives calculated for Figure 3D? I couldn't find any details. The time course shown on the Northern blots doesn't look long enough to calculate a half life accurately for some of the samples. The methods section isn't comprehensive - the papers that are referenced do not actually contain the described methods (e.g. for Northern blotting). Please include detailed methods.

4. Figure 7A,C - why is GST-NBR-2xMut larger than GST-NBR-wt?

5. In Figure EV1I-J, why doesn't GFP-Nanos interact with CCR4 or POP2? Is this reproducible? One would expect CCR4 and POP2 to be in complex with NOT1, NOT2, NOT3 and therefore co-IP.

Minor comments

6. P13, Reference for Fig 6A,B is in the wrong place (it is with structure of NOT module but should be for NOT module-NBR)

7. It would be helpful to mark the position of the additional helix (a22') on the overview figure of the structure (Fig 6A).

1st Revision - authors' response 29 January 2016

Response to Referee 1

The referee states that "In general, this study is very important to the field and also has some general interest. I therefore recommend this manuscript to be published in EMBO Journal provided the authors address my following concerns or comments (which I consider requires minor revisions)":

1. One of the main points of this study, the surprising difference between Drosophila and humans in their way to recruit CCR4-NOT can be extended a little by adding Nanos alignments between hs and dm Nanos. Especially since the NOT modules are highly conserved, even the NED binding sites on NOT are almost identical. That raises the question if there are other factors in humans, which take up the same sites as NED in Drosophila or the other way around. Can these factors be identified by simple bioinformatics analysis?

We would like to stress that the N-terminal sequences of human and Drosophila Nanos proteins that interact with the CCR4-NOT complex appear to be completely unrelated in phylogenetic terms, precluding a meaningful alignment of these sequences. It is impossible to identify a NIM sequence in Drosophila Nanos, and there is no sequence similarity to the Drosophila NBR in vertebrate or other Nanos proteins that contain a NIM. This is also the reason for using different acronyms (see the response to point 9). To identify NIM- or NBR-like sequences in other proteins we did various profile-based searches (PSI-BLAST, HHsenser as available in the MPI Bioinformatics Toolkit, see also point 10), but we could not identify any meaningful hits in proteins other than Nanos homologs. This is not too surprising given the short length and low information content of the profiles, and it suggests that competitors of the NIM or NBR peptides can only be identified using an experimental approach.

2. The authors claim that the deletion of the BoxA motif in hunchback mRNA has "no" effect on Nanos activity, thus they conclude that Nanos binds and represses the hb reporter independently of BRAT. I think, however, that in Figure 2B,C, there is a difference between WT and BoxA deletion. The difference is not large but might raise the question where this difference comes from. Is there some dependence on BRAT for Nanos RNA binding (improving RNA binding) after all, or might deletion of the 5 GUUGU's at NRE1 and NRE2 not be enough? A few weeks ago, the same authors who published the cited paper (Loedige et al., 2014), identifying the GUUGU motif have published another article (Loedige et al., 2015), where the actual and optimal BRAT binding motif has been identified. It seems to be UUGUUG. If you delete GUUGU from NRE1 there isn't something left where BRAT could bind to. However, RNA binding might be retained considerably at NRE2, where GUUGU deletion results in UUUUCG, to which BRAT should still be able to bind.

We agree with the referee that we cannot formally exclude from our experiments that BRAT still binds to the hb reporter, although we deleted the BoxA sequences. In the revised manuscript, we therefore avoid the conclusion that Nanos binds independently of BRAT and we also mention the recent publication by Loedige et al., 2015. Nevertheless, the Nanos NED has intrinsic repressive activity independently of BRAT and PUM, as shown by the tethering assays using the isolated NED (Figure 1B,C,H,I).

3. Page 10, last paragraph. Also PAN3 seems to be pulled down, comparing the input between PAN2 and PAN3? Also, it should refer to figures EV1F-L, now it refers only to figures EV1G-L.

We corrected the typo and adjusted the text to acknowledge that PAN3 is pulled down as well to some extent.

4. Page 14, bottom it says:"...adopt an SH3-like fold comprising a five-stranded half-open beta-barrel that mediates dimerization." To me, it looks as if the helical bundle mediates dimerization?

We thank the reviewer for pointing out this misleading formulation and corrected the text accordingly.

5. The description of structural details and their figures clearly needs revision. Many residues mentioned in the text are not shown in the figures. Especially the explicitly described K737-F152 carbonyl oxygen contact is not visible in the figure and hard to imagine to take place, as the side chain of K737 seems to be rather far away from the F152 carbonyl. Also, from 6E it is hard to see if there is a indeed a contact between F1876 and F130 or L127. The ring-ring interactions seem unusual anyways, maybe due to the low resolution of 3.9 Angström? It is always a pity that the coordinates are not available to the reviewers (for understandable reasons). This makes it hard to judge if the structure has been refined properly. But I trust it has, as experts in the field did it. Nevertheless, might it be worthwhile to look at the interface again?

As suggested, we carefully re-evaluated all interfaces for the presence of side-chain density, correct rotamer assignment and correct atomic distances between interacting residues. We should point out that the resolution of the complex is at 3.1Å (not at 3.9 Å as mentioned by the reviewer), where individual atom refinement is very well possible with estimated atomic coordinate errors of less than 0.422 Å. Estimated coordinate errors are now included in Table 1. Furthermore, we made sure that all of the residues from the text can now be found in at least one of the Figures or Extended Views. Finally, we modified some of the panels in Figures 6, EV4 and EV5 and added some additional panels in order to provide the reader with more comprehensive views of the structural details.

6. Should Figs 6H-J moved over to Figure 7?

We prefer to keep all structure panels in one figure, also due to considerations of space in Fig 7.

7. Can the authors comment whether they have tried further NED sequences to co-crystallize with the NOT module, which bind in a redundant manner (regions 50-115 and 164-236)? Didn't it work? If it did, why not including here?

The suggested regions bind the CCR4-NOT complex but not the NOT module (see Figure 5A, lane 14, NEDΔNBR). The corresponding peptides were therefore not tried for co-crystallization.

8. What about the affinity of the single NED regions to the NOT-module and of both together? It would be interesting to see if there is a cooperative effect or just additive.

We agree with the referee. However, our attempts to obtain quantitative data by ITC failed due to aggregation of the NOT module in the measurement cell. From the structural analysis, we would expect a clear avidity effect between the two regions, but probably no cooperative effects.

9. The addition of more and more acronyms and abbreviations does not make it easier to read. Wouldn't it be better to call them all NIMs, numbering them and put a species indicator in front? Like hsNIM1 (NIM), dmNIM2 and dmNIM3 (NED - N1BR N3BR)?

We consider it important to clearly distinguish between the phylogenetically related NIMs with their single interaction surface and the phylogenetically distinct and multi-partite NEDs. We therefore decided to keep our acronyms.

10. The authors should very briefly say how the alignments were done and what program was used to display them (at least one citation is therefore missing).

The sequences were retrieved from TREEFAM (http://www.treefam.org) and aligned using the MAFFT webserver (http://mafft.cbrc.jp; L-INS-i preset) from within JALVIEW (http://www.jalview.org). Positional conservation scores were calculated using the SCORECONS webserver (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/valdar/scorecons_server.pl) with default settings. We have added this information and corresponding citations in the Supplementary Materials and Methods in the Appendix.

Response to Referee 2

The referee states that: “Here, Raisch and colleagues present a beautiful paper that sheds important light on how Drosophila Nanos interacts with both NOT1 and NOT3 subunits of the CCR4-NOT complex in a manner that is unique to invertebrates. The paper is well-written, and the data are of high quality and novel. This paper should definitely be published in EMBO”.

Minor points:

1. On page 11, the authors write 'Deletion of the NOT1 C-terminal region that includes the SHD domain reduced but did not abolish the interaction of Nanos with NOT1 (FIG EV2G, lane 12)'. Considering that GFP-Nanos was not expressed or IP'd as efficiently as in other lanes, I'm not completely convinced that there is any reduction of this mutant in binding NOT1 whatsoever. The authors may want to mention this in their results.

We agree with the referee and have modified the text accordingly.

2. The authors should also provide a diagram depicting NOT1 and the deletion mutants that were constructed. It would be of help to the readers who may not know NOT1 structure.

A diagram depicting NOT1 is shown in Figure EV1A. The deletion mutants are described in Table S1. We now refer to Figure EV1A and Table S1 when we mention the NOT1 deletion mutants in the text.

Response to Referee 3

The referee states that: “Overall, this paper reports a number of new and interesting findings regarding RNA recruitment by CCR4-NOT and Nanos. The data are of high quality and the text is clear. This work will be an important contribution to the field”.

Specific comments

1. In Figure 3, the authors show experiments from cells where DCP2 is depleted and a catalytically inactive mutant (E361Q) is overexpressed. This approach has been used in other work but it's not clear why both depletion of wild-type DCP2 and overexpression of a catalytically inactive version are required?

We modified the text on pages 9 and 10 to clarify that an efficient inhibition of mRNA decapping requires both knockdown of the endogenous DCP2 and overexpression of a dsRNA-resistant catalytically inactive mutant. The mutant has a dominant negative effect and therefore probably works by competing with endogenous DCP2 for incorporation into endogenous decapping complexes. This competition is facilitated when the levels of endogeneous DCP2 are reduced by depletion. On the other hand, DCP2 depletion alone is not sufficient to efficiently block decapping in S2 cells (Eulalio et al., 2007. Genes & Dev 21, 2558).

2. In Figure 3A, F-luc activity should be shown as well as mRNA levels.

We now show the F-Luc activity values in Figure EVx.

3. How were the mRNA half-lives calculated for Figure 3D? I couldn't find any details. The time course shown on the Northern blots doesn't look long enough to calculate a half life accurately for some of the samples. The methods section isn't comprehensive - the papers that are referenced do not actually contain the described methods (e.g. for Northern blotting). Please include detailed methods.

We have included a detailed description in the Supplemental Materials and Methods in the Appendix.

4. Figure 7A,C - why is GST-NBR-2xMut larger than GST-NBR-wt?

We should point out that all constructs used in this study were confirmed by sequencing. We therefore assume that the addition of the double negative charge (two aspartates) alters the electrophoretic mobility of the mutant. We added a note to the legend.

5. In Figure EV1I-J, why doesn't GFP-Nanos interact with CCR4 or POP2? Is this reproducible? One would expect CCR4 and POP2 to be in complex with NOT1, NOT2, NOT3 and therefore co-IP.

In this Figure we performed coimmunoprecipitation assays using overexpressed recombinant proteins. Therefore, it is likely that HA-tagged POP2 and CCR4 are in excess relative to the endogenous CCR4-NOT complex and are not quantitatively incorporated into endogenous complexes.

Minor comments

6. P13, Reference for Fig 6A,B is in the wrong place (it is with structure of NOT module but should be for NOT module-NBR).

We agree with the referee. Fig 6A,B is now cited when we first mention the NOT module-NBR complex.

7. It would be helpful to mark the position of the additional helix (α22') on the overview figure of the structure (Fig 6A).

We have labeled helix α22' in Figure 6A and EV4A.

2nd Editorial Decision 08 February 2016

Thank you for submitting the revised version of your manuscript. It has now been seen by one of the original referees (comments shown below).

As you will see the referee finds that all criticisms have been sufficiently addressed and I am therefore happy to inform you that your study has been accepted for publication in The EMBO Journal.

REFEREE REPORT

Referee #1:

The authors have satisfactorily addressed my concerns and the comments by the other referees. I therefore recommend this manuscript to be published in 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: Elisa Izaurralde
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2015-93634

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 $\chi 2$ 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.

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?	NA. This manuscript does not include statistical analyses.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA. This study does not include any animal experiments
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA. We did not apply any formal rejection criteria.
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. We did not define explicit randomization procedures. To the best of our knowledge, we can exclude any systematic error in data analysis.
For animal studies, include a statement about randomization even if no randomization was used.	NA. This study does not include any animal experiments
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. We did not take any explicit measures, but data analysis was done in teams of at least two investigators.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA. This study does not include any animal experiments
5. For every figure, are statistical tests justified as appropriate?	NA. This manuscript does not include statistical analyses.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA. This manuscript does not include statistical analyses.
Is there an estimate of variation within each group of data?	NA. This manuscript does not include statistical analyses.
Is the variance similar between the groups that are being statistically compared?	NA. This manuscript does not include statistical analyses.

C- Reagents

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

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

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).	Antibodies are listed with catalog numbers or literature reference in Appendix Table S2.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293T and S2 cells were purchased at ATCC and are regularly checked for mycoplasma contamination.

* 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. This study does not include any animal experiments
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA. This study does not include any animal experiments
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. This study does not include any animal experiments

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA. This study does not include any human subjects.
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. This study does not include any human subjects.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA. This study does not include any human subjects.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA. This study does not include any human subjects.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA. This study does not include any human subjects.
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. This study does not include any human subjects.
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. This study does not include any human subjects.

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	Macromolecular structure data has been deposited under the following PDB accession codes: 5FU6: NOT module of the human CCR4-NOT complex (Crystallization mutant) 5FU7: Drosophila Nanos NBR peptide bound to the NOT module of the human CCR4-NOT complex See page 26.
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).	Anomalous data structure factors for the selenomethionine-substituted NOT module-Nanos complex are available as Source data with this article.
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. This study does not include any clinical or genomic datasets
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	NA. A separate Data Availability section is not necessary.
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.	NA. This study does not include any computational models

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.	NA.