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JOURNAL

Molecular architecture of LSM14 interactions involved in the assembly of mRNA silencing complexes

Tobias Brandmann, Hana Fakim, Zoya Padamsi, Ji-Young Youn, Anne-Claude Gingras, Marc R. Fabian and Martin Jinek.

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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 29th August 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, all referees express interest in the findings reported in your manuscript and highlight the technical quality of the work. However, they also raise concerns about the functional and biological implications of the structures presented and ask that this aspect of the study is extended before they can support publication of the manuscript here. In particular, ref #3 makes constructive suggestions for adding more insight on the role for LSM14 in P body formation and translational repression, while ref #2 wants to know more about the relative interplay between other known LSM14 binders, both at a structural and dynamic scale.

Given the referees' overall 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.

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

REFEREE REPORTS

Referee #1:

LSM14 is the key protein in the assembly of the mammalian gene silencing complexes through interactions with the eIF4E-binding protein 4E-T and the DEAD-box RNA helicase DDX6, but the molecular basis underlying these interactions remains elusive. In this manuscript, Brandmann et al. determined the crystal structure of the LSM domain of LSM14 in complex with the C-terminal region of 4E-T and showed that the C-terminus of 4E-T wraps around the LSM domain of LSM14 via a bi-partite motif. Subsequent binding assays in vitro and in vivo validated the importance of the residues involved in the LSM14-4E-T interface. The authors also used in vitro binding assays to show that LSM14 uses its FDF and TFG motifs rather than the conserved FFD motif to interact with the C-terminal

RecA like domain of DDX6 (DDX6c). They then solved the structure of DDX6c bound to the C. elegans LSM14 ortholog Car-1 containing the FDF, FFD and TFG motifs. The structure of DDX6c-Car-1 showed that LSM14 uses conserved non-contiguous FDF and TFG motifs to bind DDX6 with the FFD motif being dispensable for this interaction. Mutagenesis combined with P-body localization experiments indicated that the integrity of the DDX6-LSM14 interaction is required for P-body assembly. Importantly, they demonstrated that the role of LSM14 FFD motif is to recruit the decapping activator EDC4 in the formation of the mRNA silencing complexes and P-body assembly. Overall, this study nicely illustrates how LSM14 interacts with DDX6 and 4E-T in the assembly of the mRNA silencing complexes and broadens our knowledge in the field of mRNA decay and translational repression.

Minor comments:

(1) Page 13, first line: "for" appears to be a typo and should be deleted.
(2) Crystallographic Table: Decimal digits are not consistent, for example, both two and three decimal digits in resolution are used.
(3) Page5, second paragraph. The authors showed that a central region of 4E-T is also involved in the binding to LSM14 but they only used the C-terminus of 4E-T for structure determination. Is there any reason for just using the C-terminus of 4E-T for this study? Would the central region of 4E-T interact with LSM14 in a manner similar to that of the C-terminus?

Referee #2:

The regulation of gene expression involves a large number of proteins that interact in a highly dynamic fashion. This warrants the degradation of the mRNA transcript, the silencing of gene-expression and the localization of the mRNA degradation factors to processing bodies. The details of these interactions are often not known, especially because the linear motifs that are involved evolve fast and differ between different species.

In their manuscript, Jinek and colleagues, study how the LSM14/RAP55 protein is integrated in the gene expression interaction network. To that end, they solved the structures of the LSM14 protein in complex with the 4E-T and in complex with DDX6. The authors find that the LSM domain of LSM14 interacts with a conserved C-terminal fragment of 4E-T that adopts a mainly helical conformation in the complex. The FDF and TGF motifs in the C-terminal region of LSM14 form a helical structure upon interaction with the C-terminal RecA-like domain of DDX6. The structures are validated using structure-based mutations that abolish the observed interactions and with in-vivo and in-vitro pull-down experiments. Finally, the authors identify a FFD motif in LSM14 that interacts with the EDC4 protein.

The manuscript is well written and presents interesting structural data. The biological insights remain, unfortunately, somewhat limited, which might be of a concern for the EMBO journal.

I have the following remarks to the manuscript:

The solved structures should have been compared more thoroughly with known structures LSM domains in complex with ligands/ known structures of RecA domains in complex with ligands. Especially, close comparisons with the structures of Fromm et al, 2012; Tritschler et al, 2007 respectively Tritschler et al, 2009 should be made. How different are the presented structures form the known structures?

Page 10: "P-body formation was disrupted in both cases, suggesting that the interaction between LSM14 and DDX6 is required for de novo P-body formation (Fig 5C)." I don't agree with this conclusion, to assess if P-bodies are still intact one would need to observe the localization of Dcp2 in addition. The fact that LSM14 and DDX6 are no longer localizing to foci does not mean that processing bodies are gone. It is likely that processing bodies that lack those two proteins are formed.

To better understand how the presented interactions compete with known interactions, (relative) affinities should be determined. E.g. can other FDF and FDF-like motifs compete with the interaction between LSM14 and DDX6. This information is important to be able to obtain insights into the network of interactions that regulate gene-expression.

Referee #3:

The manuscript reports two crystal structures that shed light on the mechanism of how mRNA silencing complexes

containing Lsm14, DDX6 and 4E-T are assembled in cells.

First, the authors determine a 2.6 Å structure of the SM like (Lsm) domain of Lsm14 in complex a minimal interacting region of 4E-T. The Lsm domain is a globular and recognized on separate surfaces in a bipartite manner by tandem short linear motifs found in the C-terminus of 4E-T (4E-Tc). Though a domain swapped dimer mediated by this motifs is observed in the crystal, the authors present a model of the interaction that is well supported by SEC-MALS indicating the complex is a monomer; moreover, lesions in the bipartite motif or the surface reported in the aforementioned model abrogate binding assayed by pull-down assays in vitro or co-immunoprecipitation from cells where Lsm14 has been depleted by RNAi and complemented with resistant constructs.

Second, the authors biochemically map interactions between Lsm14 and the DEAD-box ATPase DDX6. A bipartite motif in Lsm14 is identified which is critical for the interaction the C-terminal REC-A domain of DDX6 in vitro and in cells (DDX6-C). The first part of this motif is comprised of amino acids FDF, which is found in other DDX6 interaction partners that promote translational repression (Scd6, Tra1) and decapping (Edc3) or both (Pat1). The second motif is comprised of amino acids TFG and is strictly required for the interaction with DDX6 in cells and in vitro. A third, intervening FFD motif is dispensable for Lsm14 binding to 4E-T. The authors determine the crystal structure human DDX6-C in complex with the cognate (FDF ...TFG) interaction motif of C. elegans Lsm14 to 3.0 Å resolution. These studies reveal that both motifs bind the DDX6-C domain, which echos structural studies of the budding yeast DDX6 ortholog (Dhh1) with Pat1. However, the authors observe that the order the motifs is reversed in Lsm14 -DDX6-C complex relative to the same motifs Pat1 bound to Dhh1-C. Intriguingly, the affinity-purification coupled with mass-spectrometry analysis of Lsm14 containing or lacking the intervening FFD motif reveals it is critical for binding the mRNA decapping activator Edc4. This new connection between Lsm14 and Edc4 was confirmed by immunoblot analysis and by localization experiments showing the FFD motif of Lsm14 is required for its localization to mRNA processing bodies (but not stress granules).

In all, a new link between the decapping and silencing complex (of 4E-T, Lsm14 and DDX6) is supported by biochemical, localization and structural data. This study provides potentially important insights but the functional significance Lsm14 interactions with Edc4 for mRNA decay requires clarification. The article would be acceptable for publication if the following points were addressed:

Major:

1-What happens to repression of mRNA translation or RNA stability when the FFD motif of Lsm14 is deleted?

2-How does the C-terminus of 4E-T, which interacts with Lsm14, contribute to mRNA stability or translational repression?

Tethered function assays are well within the expertise of these authors and could address both points above.

Minor:

3-DDX6 makes multiple contacts with the aforementioned repressive mRNP as it interacts with Lsm14 and 4E-T. It would be helpful if the authors discussed if interactions occur simultaneously or sequentially in a common pathway to promote repression and decay.

1st Revision - authors' response 17th December 2017

We wish to thank the reviewers for their thoughtful and constructive comments on our manuscript. In response to the Reviewers' recommendations and requests, we have performed additional experiments, provided additional novel functional data, and revised the text in light of their suggestions, as detailed below.
Our response to the specific comments of the Reviewers is detailed below.
We trust that we have responded satisfactorily to the criticisms and that the manuscript is now acceptable for publication in the EMBO Journal.

Reviewer #1

Overall, this study nicely illustrates how LSM14 interacts with DDX6 and 4E-T in the assembly of the mRNA silencing complexes and broadens our knowledge in the field of mRNA decay and translational repression.
We appreciate the Reviewer's positive response to our manuscript.

Minor comments: 1) Page 13, first line: "for" appears to be a typo and should be deleted.
This typo has now been corrected in the revised manuscript.

2) Crystallographic Table: Decimal digits are not consistent, for example, both two and three decimal digits in resolution are used.

The crystallographic Table has been revised. Numerical values for statistics commonly expressed as percentages are now consistently quoted to three significant figures (single decimal). I/sigmaI and B-factors are likewise quoted to a single decimal. Wavelengths are quoted to five decimal figures due to the precision with which the wavelength was set during data collection. Unit cell edges and resolution limits are quoted to the nearest 0.01 Å, as reported by the data processing software (XDS) and recorded in the mtz files that have been submitted to the PDB.

3) Page 5, second paragraph. The authors showed that a central region of 4E-T is also involved in the binding to LSM14 but they only used the C-terminus of 4E-T for structure determination. Is there any reason for just using the C-terminus of 4E-T for this study? Would the central region of 4E-T interact with LSM14 in a manner similar to that of the C-terminus?

We have attempted to crystalize a complex of the central region of 4E-T with the LSM14 N-terminal LSM domain, but have not been successful so far. Nevertheless, we believe that the central region of 4E-T interacts with LSM14 in a manner similar to that of the 4E-T C-terminus. This is based on the observations that (i) the middle and C-terminal regions contain conserved hydrophobic residues that can be partially aligned (Fig EV1A),
(ii) both the 4E-T central and C-terminal regions directly bind to the LSM14 N-terminal LSM domain (Nishimura et al., 2015) and (iii) our LSM14 N-terminal domain mutant, which does not interact with the 4E-T C-terminus, also does not co-immunopreciptiate endogenous full-length 4E-T. Collectively, these results suggest that both the middle and C-terminal regions of 4E-T interact with the same binding site in LSM14LSM, which implies that the two motifs in 4E-T have redundant functions and/or that they serve to ensure binding LSM14 with a 1:2 stoichiometry. We now include these interpretations in the Discussion section.

Reviewer 2
1. The solved structures should have been compared more thoroughly with known structures LSM domains in complex with ligands/ known structures of RecA domains in complex with ligands. Especially, close comparisons with the structures of Fromm et al, 2012; Tritschler et al, 2007 respectively Tritschler et al, 2009 should be made. How different are the presented structures form the known structures?

In response to Reviewer's request, we have now added more extensive comparisons of the presented structures with already available structural data. In the case of the LSM domain of LSM14 we note that despite an overall high structural similarity between the LSM domains of LSM14 and EDC3 and canonical Sm domain proteins such as SmD3 or LSM1-7, there are critical differences. LSM14 and EDC3 lack a N-terminal helix, which contributes to multimerization in canonical LSM proteins. These features may explain the observations that LSM14 and EDC3 do not multimerize, presumably because loss of oligomerization has accompanied the evolution of interaction surfaces for their respective interaction partners. These points are now discussed on p. 6.

Furthermore, comparison of our DDX6C-LSM14FDF-TFG structure with structures of DDX6 in complex with EDC3, 4E-T and Pat1 (Ozgur et al, 2015; Sharif et al, 2013; Tritschler et al, 2009) reveal that all four DDX6C-binding proteins use similar, conserved sequence motifs to occupy the same binding sites on DDX6, implying that their binding is mutually exclusive (Fig EV3B). However, in contrast to the other DDX6-interacting proteins (EDC3, 4E-T and Pat1) the DDX6 interacting motifs in LSM14 are noncontiguous and adopt an inverted arrangement. Although this results in weaker binding (see response to point 3), the arrangement is required to mediate additional interactions with EDC4 through the intervening FFD motif. These structural comparisons of the various DDX6C-binding motifs are now extensively discussed in the Results section (p. 13) and in the Discussion (p. 16).

2. Page 10: "P-body formation was disrupted in both cases, suggesting that the interaction between LSM14 and DDX6 is required for de novo P-body formation (Fig 5C)." I don't agree with this conclusion, to assess if P-bodies are still intact one would need to observe the localization of Dcp2 in addition. The fact that LSM14 and DDX6 are no longer localizing to foci does not mean that processing bodies are gone. It is likely that processing bodies that lack those two proteins are formed.

We appreciate the Reviewer's concern but note that the originally submitted manuscript already included immunofluorescence data as a supplemental figure showing localization of DCP1, which is a *bona fide* P-body marker (Kedersha and Anderson, 2007). These data (now presented as Fig EV3A) clearly show that DCP1 foci are present in cells expressing wild-type LSM14, but are disrupted when cells were complemented with LSM14 mutants that cannot bind DDX6. We have clarified the text in the revised manuscript to make sure that this supplemental figure is explicitly referred to.

3. To better understand how the presented interactions compete with known interactions, (relative) affinities should be determined. E.g. can other FDF and FDF-like motifs compete with the interaction between LSM14 and DDX6. This information is important to be able to obtain insights into the network of interactions that regulate gene-expression.

In order to obtain insights in the dynamic network of interactions impinging upon DDX6, we determined the affinities of LSM14, EDC3, 4E-T and PATL1 for DDX6 quantitatively using ITC (Table2, Fig EV1F and EV4). Analysis of the binding isotherms shows that the interaction of LSM14 with DDX6 occurs with a Kd of 1.62 μM, about 5-fold weaker compared to PATL1, EDC3 and 4E-T (Kd ~0.23–0.41 μM). We hypothesize that this decreased affinity reflects the unique mode of interaction that is employed by LSM14 to bind DDX6. Furthermore, the inverted arrangement of the DDX6 interacting motifs in LSM14 could assist in presenting its FFD motif in a way that facilitates its association with EDC4. Nevertheless, given that the affinities of all interaction motifs of the DDX6 partners fall within an order of magnitude of one another, it is likely that they can all compete with each other, given that the binding of a specific interacting partner to DDX6 will also be dependent on the relative abundance of each factor. Quantitative proteomic studies have estimated the cellular concentration of DDX6 in HeLa cells to be ~550 nM, approximately equal to the combined concentrations of LSM14 (LSM14A: 235 nM and LSM14B: 94 nM), 4E-T (10 nM), EDC3 (93 nM) and PATL1 (100 nM) (Hein et al, 2015). This suggests that in a cellular context, DDX6 is thus sufficiently abundant to accommodate all its interaction partners. Additionally, direct interactions with DDX6 will also be likely modulated by the presence of other, indirect interactions. For example, 4ET, which directly interacts with DDX6, also recruits LSM14, which in turn would compete with 4E-T for binding to DDX6. Moreover, the interactions are almost certainly influenced by the propensity DDX6 and many of its interacting partners to undergo phase separation and liquid droplet formation, which in turn may have an effect on the local concentration. As a result, it is not clear whether one can infer a specific sequence of binding events that would underpin DDX6-dependent translational repression and decay.
We additionally quantified the binding of the LSM14LSM domain for the C-terminal region of 4E-T, showing that the equilibrium dissociation constant for the LSM14LSM– 4E-TC complex is ~0.3 μM. In contrast, the binding of the W985A LSM14LSM protein to 4E-TC was not detectable.

Reviewer 3

1. What happens to repression of mRNA translation or RNA stability when the FFD motif of Lsm14 is deleted?

We have used a lN-BoxB tethering assay to test the contributions of the FFD motif to reporter mRNA stability. We observe that deleting the FFD motif from LSM14 did not impact its repressive capacity in tethering assays (data not shown). This is not completely unexpected, as our proteomics data indicate that LSM14 interacts with a multiple protein partners that additionally interact with each other independently of LSM14. Thus, abolishing the LSM14-EDC4 interaction may not be enough to impact silencing if LSM14 is still interacting with additional binding partners, including 4E-T and DDX6. Nevertheless, our data clearly demonstrate that the FFD motif plays an important role in LSM14 for promoting its interactions with other mRNP repressive factors in higher-order RNP complexes (P-bodies) in cells.

2. How does the C-terminus of 4E-T, which interacts with LSM14, contribute to mRNA stability of translational repression?

We now include new functional data using tethering assays, where we tether the LSM14 N-terminal LSM domain to a reporter mRNA (Fig 2F). Tethering lNHA-tagged wild-type LSM domain repressed our Renilla luciferase (RL) 5BoxB reporter ~3-fold when compared to tethering a control protein (LacZ). This is in keeping with a previous study that tethered the *Xenopus* ortholog of LSM14 to a reporter in oocytes, and found that the repressive capacity of LSM14 resides in its N-terminal half (Tanaka et al., 2006). In contrast, tethering our mutant LSM domain, which does not interact with 4E-T, does not efficiently silence our reporter (~1.2-fold). These data therefore suggest that LSM14 requires 4E-T contact in order to efficiently repress gene expression of a target mRNA. Moreover, these results further support our conclusion that both the middle and C-terminal motifs in 4E-T interact with the LSM14 LSM domain via a similar binding mechanism.

References:
Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, Toyoda Y, Gak IA, Weisswange I, Mansfeld J, Buchholz F, Hyman AA, Mann M (2015) A human interactome in three quantitative dimensions organized by stoichiometries and abundances. *Cell* **163:** 712-723
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Ozgur S, Basquin J, Kamenska A, Filipowicz W, Standart N, Conti E (2015) Structure of a Human 4E-T/DDX6/CNOT1 Complex Reveals the Different Interplay of DDX6Binding Proteins with the CCR4-NOT Complex. *Cell reports* **13:** 703-711
Sharif H, Ozgur S, Sharma K, Basquin C, Urlaub H, Conti E (2013) Structural analysis of the yeast Dhh1-Pat1 complex reveals how Dhh1 engages Pat1, Edc3 and RNA in mutually exclusive interactions. *Nucleic acids research* **41:** 8377-8390
Tanaka KJ, Ogawa K, Takagi M, Imamoto N, Matsumoto K, Tsujimoto M (2006) RAP55, a cytoplasmic mRNP component, represses translation in Xenopus oocytes. *Journal of Biological Chemistry* **281**:40096-106.
Tritschler F, Braun JE, Eulalio A, Truffault V, Izaurralde E, Weichenrieder O (2009) Structural basis for the mutually exclusive anchoring of P body components EDC3 and Tral to the DEAD box protein DDX6/Me31B. *Molecular cell* **33:** 661-668

2nd Editorial Decision 4th January 2018

Thank you for submitting a revised version of your manuscript to The EMBO Journal. It has now been seen by two of the original referees and their comments are shown below.

As you will see the referees both find that all criticisms have been sufficiently addressed and they support publication here. However, before we can go on to officially accept the manuscript there are a few editorial issues concerning text and figures that I need you to address in a final revision:

-> Please include the running title in the manuscript text file

-> Please provide 3-5 keywords for your study. These will be visible in the html version of the paper and on PubMed and will help increase the discoverability of your work.

-> Please update the reference style in the manuscript to fit with the journal guidelines (one author name + et al listed in the main text; 20 authors names listed before 'et al' in the reference list)

-> I noticed that the two fields for 'Reagents' were left empty in the author checklist that you provided. While the information on cell lines and antibodies used can be found in the manuscript file we would prefer to have it listed here as well (to allow easier access for the reader). I would therefore ask you to include an updated version of the checklist.

-> Our format allows up to 5 figures to be displayed at Expanded View figures (typeset and in line with the main manuscript in the html version) and I noticed that your manuscript currently has 7. I would therefore ask you to either combine some of these figures (eg EV2-EV3 and EV6-EV7) or move two of them to an Appendix file. Please make sure to update the callouts in the manuscript text file accordingly. Feel free to contact me questions about this.

-> Please make sure to include a scale bar in all IF images (figs 5, EV3, EV6) and state the size of the bar in the legend

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to receiving your final revision.

REFEREE REPORTS

Referee #2:

The authors have fully addressed my concerns. In addition, the concerns of the other reviewers have been well addressed. I support publication of the revised manuscript.

Referee #3:

The authors have addressed my questions; the manuscript is suitable for publication in EMBOJ.

2nd Revision - authors' response 19th January 2018

I am writing to let you know that we have resubmitted the final version of the manuscripts in which we have incorporated your suggestions.

Please let us know whether everything is OK or whether you would require any additional materials/information.

Thank you for handling our manuscript.

Accepted 21st January 2018

Thank you for submitting the final version of your manuscript, I am pleased to inform you that it has now been officially 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: Dr. Martin Jinek, Dr. Marc Fabian
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2017-97869

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?	Each experiment (ITC mesurement for a given protein interaction pair) was repeated at least three times using the same stock of purified protein (biological sample) that was independently diluted at the appropriate concentrations for each of the three experiments. Tethering assays were performed in triplicate using cells from three independent transfections.
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.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes.
Is there an estimate of variation within each group of data?	NA

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Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Rabbit polyclonal against PATL1 (cat. no. A303-482A), DDX6 (A300-460A), EDC4 (A300-745A), all rom Bethyl laboratories); and LSM14 (cat. no. ABE37, Millipore). Mouse monoclonal against β-actin and FLAG (Sigma). Rabbit polyclonal antibody against 4E-T (cat. no. ab55881, Abcam).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Escherichia coli BL21 (DE3) Rosetta 2 strain (cat. No. 71400, Novagen, Merck Millipore): recombinant protein expression. Human HEK293T cell line: production of shRNA lentiviruses for gene silencing. Human HeLa cell line: coimmunoprecipitation experiments, lambdaN-BoxB tethering assays and MS proteomic analysis.

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	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	X-ray crystallographic data (atomic coordinates and structure factors) have been deposited to the Protein Data Bank under acession codes 6F9W (LSM14-4E-T- complex) and 6F9S (LSM14-DDX6 complex). Mass-spectrometry data and associated analysis files to MassIVE (Mass Spectrometry Interactive Virtual Envrionment), and to Proteome Exchange - European database. MassIVE files (Brandmann_LSM14) can be accessed through this private URL link (ftp://MSV000081830@massive.ucsd.edu) using password: LSM14A. MassIVE ID is MSV000081825. Proteome Exchange ID is PXD008505.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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