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The AAA+ ATPase TRIP13 remodels HORMA domains through N-terminal engagement and unfolding

Qiaozhen Ye, Dong Hyun Kim, Ihsan Dereli, Scott C. Rosenberg, Goetz Hagemann, Franz Herzog, Attila Tóth, Don W. Cleveland, and Kevin D. Corbett

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Thank you for submitting your manuscript on HORMA domain remodeling by TRIP13 for our editorial consideration. It has now been seen by two expert referees, and I am happy to inform you that both of them consider this work well-executed and important. They do raise a number of specific issues, which I hope should be straightforward to address during a revision of this manuscript. Pending adequate revisions, we shall be pleased to accept the manuscript for publication in The EMBO Journal.

REFEREE REPORTS

Referee #1:

This is a lovely manuscript that sheds light on the mechanism of the interaction of the AAA ATPase Trip13 and its co-factor p31comet with the HORMA domain containing protein Mad2. By combining a variety of structural, biochemical, and biophysical methods, the authors identify the N-terminal region of Mad2 as a crucial target of the ATPase required to convert closed Mad2 to open Mad2, a reaction that may be required for regeneration of the pool of open Mad2 required for sustained checkpoint signaling. Besides testing the effects of N-terminal deletion mutants of Mad2 on the checkpoint response in vivo, with results that confirm the importance of this region for the Mad2 conversion, the authors go on to confirm the generality of their conclusions on a second HORMA domain protein, HORMAD1. They then extend this analysis to other HORMA domain proteins and predict that Rev7 and Atg13 may not be targets of Trip13 as they lack a network of hydrogen bonds that the authors implicate in the stabilization of closed Mad2.

Besides the few points discussed below, I feel that the manuscript meets all the standards required for publication in EMBO Journal. The work is excellent, thorough, and well illustrated. The methodological pipeline and detailed mechanistic insight reported in the manuscript have the potential to become a reference for researchers on a rather broad front, ranging from the SAC to AAA ATPases.

Specific points:

-Introduction: I would like to ask the authors to consider giving credit to work that they partly cite - and not too precisely - and partly forgot to cite. Part of that work was even published in the EMBO Journal. In particular, neither the term 'safety belt', nor the terminology 'open' and 'closed' Mad2, nor the realization that Mad2 dimerization involves open and closed conformers were ever proposed in the three Luo et al. papers discussed early in the Introduction together with a review by the authors. Rather, these terms and concepts were respectively introduced by Sironi et al. 2002 and by De Antoni et al. 2005. The authors cite the first of these papers (not the second) but later and in a different context, and to me (A. Musacchio) it seems that they could find their home at this point, at risk of making the argument come across as being exclusively pro domo mea. Also, the interaction of p31comet with C-Mad2 was the main subject of Mapelli et al. EMBO J 2006, which the authors do not elect to cite. Finally, the characterization of the deltaN15 mutant as a binder of Cdc20/closure motifs is described already in Mapelli et al. Cell 2007 and is not a new finding of this paper.

-Page 7: 'Data' is plural

-Page 11: As originally shown by Fang, Yu and Kirschner in their 1998 G&D paper, wild type Mad2 in the absence of closure motifs forms oligomers that were later shown to consist of open and (empty) closed Mad2. This dimerization likely influences the equilibrium of the open and closed forms. The Yu laboratory also proposed the existence of a C-Mad2 dimer, whose functional and structural significance has however remained somewhat elusive. I do not have a strong opinion on the question of which between the open and closed Mad2 conformer is more stable, but I think that this is an element of consideration before concluding that empty closed Mad2 is the more stable conformer. Is it clear that the high salt peak from the ion exchange column does not contain this oligomer originally described by the Kirschner lab? Or can the authors conclude that it is pure empty C-Mad2?

Figure 3: What are the faster migrating bands detected by the anti Mad2 antibody and observed with the 17C and 106C mutants?

Referee #2:

Activated by improper kinetochore-microtubule attachment, the spindle assembly checkpoint halts the metaphase-to-anaphase transition, providing time for error correction to maintain chromosomal stability. The activation of the checkpoint results in the protein MAD2 changing from 'open' to 'closed' conformation, which is then incorporated into protein complexes that inhibit mitosis progression. Once the kinetochore-microtubule attachment error is corrected, the AAA-ATPase TRIP13 works with its binding partner p31comet to convert C-MAD2 back to O-conformation and meanwhile disassemble the inhibitory complexes. How this conversion is achieved mechanistically is unclear and is addressed in this manuscript.

The authors previously have solved the crystal structure of C. elegans TRIP13 (Pch2) and performed biochemical assays confirming the role of TRIP13 and p31comet in MAD2 C-O conversion. In this manuscript they first obtained a crystal structure of human TRIP13 hexamer, and then mapped potential binding sites of MAD2 and p31comet on TRIP13 through cross-linking and modeling. Their results suggested that the disordered MAD2 N-terminus is engaged by the TRIP13 "pore loops". Further experiments indicated MAD2 N-terminus is required for MAD2 conformational change and consequently its checkpoint activity. They also suggested that engaging with a small fragment at the N-terminus might be sufficient for inducing unfolding/refolding of large fragment of MAD2 protein to change its conformation. They extended the result to HORMAD1, another member of HORMA domain containing proteins and an important meiosis regulator, and suggested that the N-terminus of HORMAD1 might also be important for its extraction from meiotic chromosomes by TRIP13.

Overall the experiments are well designed, the results are clear and well-presented. They have carried on the tradition of nice integration of structural and biochemical analyses for the TRIP13 working mechanism, and added some functional assays in cells through microscopic imaging. As the MAD2 conformational change (both O-C and C-O) is the key to a productive and faithful mitosis, the insights garnered from the work on how the conformation remodeling occurs are valuable. Any extension to other HORMA domain proteins is a plus. I do have two major points that need to be clarified (#1) and a few minor points for the authors' attention.

1. Structure of TRIP13: does every subunit in the TRIP13 hexamer obtained in the presence of ATP bind to ATP? Does the helical configuration provide any explanation that each TRIP13 hexamer binds only one p31:MAD2 complex? What does it mean when the authors say "Crystallization trials with TRIP13:p31:MAD2 complex yielded only crystals of TRIP13E253Q alone"? It seemed that p31 and MAD2 are present in the crystals but not producing any signals (Materials and Methods). Does that mean the two proteins are probably not binding at the same subunit of TRIP13? It would be puzzling if the two proteins' presence did not even change the NTD conformation (meaning no flipping "upward" as they suggested later? But the HDX-MS indicated even p31 alone could provide some protection to a "pore loop" region on TRIP13). They previously used a dimerization defective MAD2 (R133A) but did not state in this manuscript whether this is still the case. What is the purpose to do the modeling of a "closed" TRIP13 hexamer (Fig 1F)? What is the basis that this model instead of the real crystal structure used in interpreting the crosslinking data (p6, Fig 2F)?

2. Cysteine crosslinking result (Fig 3): The reasoning behind the conclusion that "MAD2 Cys106 crosslinking to the pore loop of TRIP13" means MAD2 unfolding to 106 residues is not clear to me. The authors do state that MAD2 conformational change may need only partial unfolding, and the differences in O-MAD2 and C-MAD2 structures mainly concerns the N- and C-terminus not the core where C106 is situated.

3. P8, the beginning sentence of the section "TRIP13 requires the...": "TRIP13... maintains the soluble pool of O-MAD2 for MCC assembly..." (Similar statement in the beginning sentence of the next section). This could be confusing. I understand they echoed Ma and Poon's results on TRIP13 knock-out suggesting a rigid C-MAD2 conformation might not be good in maintaining the spindle assembly checkpoint, but it's not O-MAD2 that gets incorporated in the MCC. Also note that Hongtao Yu's lab recently solved the ΔN10-MAD2 structure (Hara et al PNAS, 2015).

4. Discussion: second paragraph, Figure EV12 should be EV11. I am surprised they discussed so little about the MAD2 results (thus my questions in #1), but spent two paragraphs on meiotic HORMAD proteins. Also note in rice p31comet has been identified as a component of the synaptonemal complex (Ji et al, PNAS, 2016).

5. Citations: some sources were not properly credited. For example, they did not mention at all the first report on TRIP13-p31comet interaction (Tipton et al, BMC Cell Biol, 2012) and the first report proposing that TRIP13 and p31comet work together to utilize ATP hydrolysis to power MAD2 C-O conformation change (Wang et al., JBC, 2014).

1st Revision - authors' response 30 May 2017

Please find attached a revised version of our manuscript titled “The AAA+ ATPase TRIP13 remodels HORMA domains through N-terminal engagement and unfolding,” which was previously reviewed for publication in *The EMBO Journal* with manuscript # EMBOJ-2017-97291.

We were very pleased to see that both reviewers found the work interesting and important. Both reviewers pointed out references that we had regrettably failed to cite, and for that we thank them. We have responded in detail to each of their other points in the attached response document. Overall, the changes and additions prompted by both reviewers have significantly improved the revised manuscript.

(Point by point response shown on the following pages.)

Response to Reviewers:

The AAA+ ATPase TRIP13 remodels HORMA domains through N-terminal engagement and unfolding

Qiaozhen Ye, Dong Hyun Kim, Ihsan Dereli, Scott C. Rosenberg, Goetz Hagemann, Franz Herzog, Attila Tóth, Don W. Cleveland, Kevin D. Corbett

We were very pleased to read the positive assessments of both reviewers, and thank them for their helpful comments. In particular, we apologize that in several cases, ideas and concepts were not credited to their proper sources, or key studies were inadvertently not cited. We have altered the manuscript accordingly, and below address each comment and criticism individually. All text and citations that have been altered or added are highlighted in **red text** in the revised manuscript.

Reviewer #1:

Introduction: I would like to ask the authors to consider giving credit to work that they partly cite - and not too precisely - and partly forgot to cite. Part of that work was even published in the EMBO Journal. In particular, neither the term 'safety belt', nor the terminology 'open' and 'closed' Mad2, nor the realization that Mad2 dimerization involves open and closed conformers were ever proposed in the three Luo et al. papers discussed early in the Introduction together with a review by the authors. Rather, these terms and concepts were respectively introduced by Sironi et al. 2002 and by De Antoni et al. 2005. The authors cite the first of these papers (not the second) but later and in a different context, and to me (A. Musacchio) it seems that they could find their home at this point, at risk of making the argument come across as being exclusively pro domo mea. Also, the interaction of p31 ^{comet} with C-Mad2 was the main subject of Mapelli et al. EMBO J 2006, which the authors do not elect to cite. Finally, the characterization of the deltaN15 mutant as a binder of Cdc20/closure motifs is described already in Mapelli et al. Cell 2007 and is not a new finding of this paper.

> We apologize for our incomplete citations of important MAD2-related concepts and findings in the introduction. We have added a citation of Sironi et al. *EMBO J.* 2002 to the first paragraph of the introduction, at the first mention of open and closed MAD2 conformations, along with a seminal review on MAD2 conformations and their functions (Mapelli et al. *Current Biology* 2007). We also cite two of the Luo et al. papers at that point (*NSB* 2000 and *Mol. Cell* 2002), as these are the first reported structure of open and closed MAD2 (despite not being referred to by those names in these papers), along with our recent review (Rosenberg & Corbett *JCB* 2015).
>
> Upon re-reading, it became clear to us that our originally-submitted manuscript did not provide enough detail into SAC activation and MCC assembly. We have expanded the second paragraph of the discussion accordingly, and included a number of new primary references including the De Antoni et al. (*Current Biology* 2005) paper mentioned by the reviewer.
>
> Regarding the p31^{comet}-MAD2 interaction, we have also expanded the section introducing TRIP13 and p31^{comet}, and added the Mapelli et al. *EMBO J.* 2006 reference plus two others dealing with TRIP13 (Tipton et al. *BMC Cell Biology* 2012; Wang et al. *JBC* 2014) that we had inadvertently failed to cite.

Finally, we apologize for failing to cite both Mapelli et al. *EMBO J.* 2006 and Mapelli et al. *Cell* 2007 for their characterization of the structure and CDC20-binding activity of the ΔN15-MAD2 construct. These references have been added to the revised manuscript on page 8.

Page 7: 'Data' is plural.

We have altered the sentence in question to read "Our HDX-MS data show that p31^{comet} residues 156-165…"

Page 11: As originally shown by Fang, Yu and Kirschner in their 1998 G&D paper, wild type Mad2 in the absence of closure motifs forms oligomers that were later shown to consist of open and (empty) closed Mad2. This dimerization likely influences the equilibrium of the open and closed forms. The Yu laboratory also proposed the existence of a C-Mad2 dimer, whose functional and structural significance has however remained somewhat elusive. I do not have a strong opinion on the question of which between the open and closed Mad2 conformer is more stable, but I think that this is an element of consideration before concluding that empty closed Mad2 is the more stable conformer. Is it clear that the high salt peak from the ion exchange column does not contain this oligomer originally described by the Kirschner lab? Or can the authors conclude that it is pure empty C-Mad2?

We agree that in the case of wild-type MAD2, O-MAD2:C-MAD2 dimerization likely plays a role in determining the equilibrium between open and closed MAD2 in solution, as this complex is potentially more stable than either monomeric species. We would point out that our *in vitro* assays used exclusively the MAD2 R133A mutant, which cannot stably dimerize (Sironi et al. *EMBO J.* 2001 and many others). In our purifications of MAD2 R133A, both the early-eluting from ion-exchange (O-MAD2) and late-eluting (C-MAD2) populations are exclusively monomeric when further examined by size-exclusion chromatography. Thus, we are confident that the late-eluting species does in fact represent pure, empty, monomeric C-MAD2 R133A. Moreover, both the Yu lab and our earlier tests with MAD2 R133A (Luo et al. *NSMB* 2004; Ye et al, *eLife* 2015) showed that, when incubated at room temperature or 37°C, MAD2 R133A is eventually converted to ~90% C-MAD2, regardless of whether it started as O-MAD2 or C-MAD2. Regardless of the pathway of conversion and the potential role of O-MAD2:C-MAD2 dimerization in this pathway, we feel that this is strong evidence that (at least for MAD2 R133A) the C-MAD2 conformer is more stable in solution, even in the absence of a closure motif/MIM. Nonetheless, in recognition of the complexity of this issue, we have removed several references to C-MAD2 as the "more thermodynamically-stable" state of MAD2 from the manuscript.

Figure 3: What are the faster migrating bands detected by the anti Mad2 antibody and observed with the 17C and 106C mutants?

As the full-gel images in **Appendix Figure S3** show, several bands are detected by the anti-MAD2 antibody between monomeric MAD2 at ~25 kDa, and the MAD2-TRIP13 crosslinked species migrating at ~120 kDa. It is likely that one of these (probably the species migrating at ~40 kDa) represents a MAD2-MAD2 dimer. Other species likely represent crosslinked MAD2-p31^{comet} complexes. We were unable to generate an active cysteine-free p31^{comet} construct, forcing us to use wild-type *H. sapiens* p31^{comet}. This protein contains 8 cysteine residues, several of which are predicted to be solvent-exposed (from the structure of human p31^{comet}; PDB ID 2QYF). In addition to 1:1 complexes, it is also possible that complexes containing multiple copies of either p31^{comet} or MAD2 are formed at low levels, due to the number of cysteines per p31^{comet} protomer. We have added a note to this effect in the legend to **Appendix Figure S3**.

Reviewer #2:

1. Structure of TRIP13: does every subunit in the TRIP13 hexamer obtained in the presence of ATP bind to ATP?

Our crystals of TRIP13^{E253Q}, both in the presence and absence of ATP, are in space group $P6_5$ with a single TRIP13 protomer per asymmetric unit. We see clear density for ATP in this single subunit when the crystals are grown in the presence of ATP. As to the broader question of ATP occupancy in TRIP13 hexamers in solution, we cannot confidently speculate. Our prior structure of *C. elegans* PCH-2 (Ye et al. *eLife* 2015) suggested that the active TRIP13 hexamer may have ATP bound to two subunits, ADP to another two subunits, and no nucleotide bound to the final two subunits, which adopt the more-strained "open" conformation. This state would be very difficult to capture in a crystal structure. In hexamers of TRIP13^{E253Q}·ATP, we suspect that four subunits are ATP-bound, with two subunits in the "open" conformation (see model, **Figure 1F**). This, however, is probably only one of many states adopted by this dynamic machine during catalysis.

Does the helical configuration provide any explanation that each TRIP13 hexamer binds only one p31:MAD2 complex? What does it mean when the authors say "Crystallization trials with TRIP13:p31:MAD2 complex yielded only crystals of TRIP13^{E253Q} alone"? It seemed that p31 and MAD2 are present in the crystals but not producing any signals (Materials and Methods). Does that mean the two proteins are probably not binding at the same subunit of TRIP13? It would be puzzling if the two proteins' presence did not even change the NTD conformation (meaning no flipping "upward" as they suggested later? But the HDX-MS indicated even p31 alone could provide some protection to a "pore loop" region on TRIP13).

We apologize for the confusion on these related points. First, we speculate that TRIP13 can transiently associate with several copies of p31^{comet} through its six N-terminal domains. Only one copy of p31^{comet}:MAD2, however, could be stably accommodated in a "flipped-up" conformation in which MAD2 is engaged with the hexamer pore (**Figure 2F**).

Second, we speculate that the helical configuration of TRIP13 seen in our crystals arises because the "closed" conformation of TRIP13 is a lower-energy state than the "open" conformation. In solution at relatively low concentration, the competing energetics of: (1) satisfying subunit-subunit interfaces, versus (2) protein conformation, tends to stabilize hexamers. At the extremely high protein concentrations of a crystal lattice, a helical filament forms that both satisfies the native subunit interfaces and allows all subunits to adopt the lowest-energy conformation.

When we set up crystallization trials of the TRIP13:p31^{comet}:MAD2 complex, we obtained only crystals of TRIP13 alone. There is no indication of p31^{comet}:MAD2 in the resulting electron density, and indeed the crystals do not require p31^{comet}:MAD2 to form (though in our initial trials, p31^{comet}:MAD2 appeared to function essentially as a "chaperone", helping maintain TRIP13 solubility during crystallization). We speculate that formation of the helical TRIP13 filament, and the lateral packing of filaments into a crystal, combine to dissociate p31^{comet}:MAD2 from TRIP13. Based on our rough 3D model of the complex (**Figure 2F**), this makes sense: if a

> large part of the TRIP13-substrate interface involves the top surface of the hexamer, then filament formation essentially destroys this surface, likely destabilizing substrate binding. These points are noted more fully in the revised manuscript on page 18-19 (**Materials and Methods**).

They previously used a dimerization defective MAD2 (R133A) but did not state in this manuscript whether this is still the case.

> We apologize for this omission. Initial purification of the TRIP13:p31^{comet}:MAD2 complex, crystallization trials, and XLMS/HDX-MS experiments were all done using wild-type human MAD2. This MAD2 was always used in the presence of p31^{comet}, a closure motif-containing peptide, or both, thereby minimizing the tendency of MAD2 to dimerize in these experiments. For biochemical assays, the R133A dimerization-defective mutant of both human and mouse MAD2 was used. This is more clearly noted on page 8 of the revised manuscript, and more fully explained in the **Materials and Methods** section.

What is the purpose to do the modeling of a "closed" TRIP13 hexamer (Fig 1F)? What is the basis that this model instead of the real crystal structure used in interpreting the crosslinking data (p6, Fig 2F)?

> We modelled a closed TRIP13 hexamer model essentially to show that the helical filament does, in fact, show the biologically-relevant subunit-subunit interfaces, and that the difference between closed hexamer and helical filament lies in the conformation of subunits (**Figure EV3**). The cross-linking data was in fact interpreted in the context of three successive subunits of the helical filament form (**Figure 2D** and **2F**), which is essentially the same as a "half-hexamer" in **Figure 1F**. This point is noted in the legend to **Figure 2D**.

2. Cysteine crosslinking result (Fig 3): The reasoning behind the conclusion that "MAD2 Cys106 crosslinking to the pore loop of TRIP13" means MAD2 unfolding to 106 residues is not clear to me. The authors do state that MAD2 conformational change may need only partial unfolding, and the differences in O-MAD2 and C-MAD2 structures mainly concerns the N- and C-terminus not the core where C106 is situated.

> While alternative explanations are possible, the fact that we observed cross-linking between the TRIP13 pore loops and MAD2 residues 17, 25, 40, 79, and 106 only in the presence of ATP, and not ATP-γS, strongly suggests that these cross-links form as a result of processive unfolding by TRIP13. We agree with the reviewer that unfolding of the HORMA domain core is probably not required for MAD2 conformational conversion, but we cannot rule out complete MAD2 unfolding as a possible (if minor) mechanism. We discuss these points in the first paragraph of the **Discussion**, on page 12.

3. P8, the beginning sentence of the section "TRIP13 requires the...": "TRIP13... maintains the soluble pool of O-MAD2 for MCC assembly..." (Similar statement in the beginning sentence of the next section). This could be confusing. I understand they echoed Ma and Poon's results on TRIP13 knock-out suggesting a rigid C-MAD2 conformation might not be good in maintaining the spindle

assembly checkpoint, but it's not O-MAD2 that gets incorporated in the MCC. Also note that Hongtao Yu's lab recently solved the ΔN10-MAD2 structure (Hara et al PNAS, 2015).

> We agree that the wording in both sections was somewhat confusing, and have altered the wording in both cases (pages 8 and 9). Regarding the point that O-MAD2 is not incorporated into the MCC, this is essentially a matter of semantics. The reviewer is correct that, in the MCC, MAD2 is in the C-MAD2 conformation. However, the bulk of evidence indicates that O-MAD2 is the only form that can be recruited to kinetochores and incorporated into the MCC (along with conversion to the closed conformation). Pre-existing "empty" C-MAD2, while competent to bind closure-motif peptides *in vitro*, is most likely unable to bind full-length CDC20.
>
> We are aware of two structures of ΔN10-MAD2: an NMR structure of a ΔN10-MAD2 monomer in the O-MAD2 conformation (Luo et al. *NSB* 2000), and the more recent crystal structure of an asymmetric ΔN10 O-MAD2:C-MAD2 dimer (Hara et al. *PNAS* 2015). We would point out that in both cases, the first 10 residues of MAD2 were removed but an N-terminal tag (MRGSHHHHHHGS) was present, meaning that this construct actually has a longer N-terminus than wild-type MAD2. We purified a similar ΔN10-MAD2 construct with a Ulp1-cleavable tag, and found that the tag could not be cleaved even with an extended incubation with Ulp1 (noted in **Materials and Methods**); this implies that the recognition sequence in the tag is involved in secondary structure, most likely forming a β1-like strand. Supporting this idea, some residues from the tag are ordered in both structures mentioned above (GS in the O-MAD2 monomer structure; as much as HHGS in the O-MAD2 protomers of the dimer structure). We don't include discussion of these points in the manuscript, as they likely have little bearing on the behavior of the ΔN10-MAD2 construct we generated in DLD1 cells, which has no tag. Based on MAD2 structures, however, we expect that tagless ΔN10-MAD2 can likely adopt both open and closed conformations.

4. Discussion: second paragraph, Figure EV12 should be EV11. I am surprised they discussed so little about the MAD2 results (thus my questions in #1), but spent two paragraphs on meiotic HORMAD proteins. Also note in rice p31^{comet} has been identified as a component of the synaptonemal complex (Ji et al, PNAS, 2016).

> We thank the reviewer for catching the figure reference error, and have corrected it (indeed, all EV figures have been renumbered according to *EMBO Journal* requirements). Regarding the discussion's focus on meiotic HORMADs, we feel that the focus on these proteins in this section is entirely appropriate, given the number of outstanding questions regarding TRIP13's mechanism of regulating these proteins. We also thank the reviewer for noting the recent finding that p31^{comet} localizes to the synaptonemal complex in rice meiosis; we have added a reference to, and discussion of this finding to the manuscript (page 12-13).

5. Citations: some sources were not properly credited. For example, they did not mention at all the first report on TRIP13-p31^{comet} interaction (Tipton et al, BMC Cell Biol, 2012) and the first report proposing that TRIP13 and p31^{comet} work together to utilize ATP hydrolysis to power MAD2 C-O conformation change (Wang et al., JBC, 2014).

> We apologize for inadvertently excluding these important references, which were the key works first showing TRIP13's role in the SAC, and in MCC disassembly in particular. As noted in our

response to reviewer #1, we have expanded the introduction of p31^{comet} and TRIP13 and added the noted references (page 3-4 of the revised manuscript).

2nd Editorial Decision - Acceptance 06 June 2017

Thank you for submitting your final revised manuscript for our consideration. I have now gone through your responses and revised manuscript, and I am pleased to inform you that in light of the satisfactory revisions, we have now accepted the paper for publication in The EMBO Journal!

Thank you again for this contribution to The EMBO Journal and congratulations on a successful publication! Please consider us again in the future for your most exciting work.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Kevin D. Corbett
Journal Submitted to: The EMBO Journal
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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).

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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?	For Figure 5D, the sample size (50 cells) was chosen as it is sufficient to demonstrate a significant effect between wild-type cells and those depleted for MAD2 (gray versus red data points; P-value <0.0001 in unpaired two-tailed t-test). For Figure 6B-D, the sample size (50 cells) was chosen arbitrarily as a high-enough value to accomodate natural variation in cell-cycle stage and visualization efficiency between samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Each HORMAD1 construct was electroporated into the testes of a single animal.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded.
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.	No
For animal studies, include a statement about randomization even if no randomization was used.	No randomization was used.
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.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
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?	No
Is the variance similar between the groups that are being statistically compared?	For Figure 5D - yes, except for MAD2 delta-N5, and this increase in variance is biologically important and discussed in the text.

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).	Information on primary antibodies is available on antibodypedia or the vendors' web sites: Anti-MAD2 (Bethyl #A300-301A; https://www.antibodypedia.com/gene/15732/MAD2L1/antibody/612896/A300-301A), anti-TRIP13 (Bethyl #A303-605A; https://www.antibodypedia.com/gene/8906/TRIP13/antibody/616158/A303-605A), anti-SYCP1 (Abcam #15090; http://www.abcam.com/scp1-antibody-ab15090.html), anti-GFP (ThermoFisher #A11122; https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A-11122), anti-FLAG M2 (Sigma-Aldrich #F1804; http://www.sigmaaldrich.com/catalog/product/sigma/f1804).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Flp-In T-REx-DLD-1 parental cells were originally obtained from the lab of Stephen Taylor, and are regularly tested for mycoplasma contamination. These cells have recently been whole-genome sequenced and karyotyped (Nature Cell Biology 19(1):68-75).

* 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.	Mus musculus, CD1, male 13 dpp, wild-type animlas were used. Animals were bred and maintained under pathogen-free conditions at the Experimental Center of the Medizinisch-Theoretisches Zentrum of the Medical Faculty at the Dresden University of Technology according to approved animal welfare guidelines. Briefly, animals were kept under 12 hour dark/light cycle, fed ad libitum and water was available all the time. Breeding age male and female CD1 mice were ordered from Charles River.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animals were used and maintained in accordance with the German Animal Welfare legislation ('Tierschutzgesetz'). All procedures pertaining to animal experiments were approved by the Governmental IACUC ('Landesdirektion Sachsen') and overseen by the animal ethics committee of the Technische Universität Dresden (license number DD24-5131/207/18).
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.	Confirmed

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 reported crystal structures can be accessed at the wwPDB (http://www.rcsb.org) under accession numbers 5VQ9 and 5VQA, as noted in the "Data Availability" section. Primary x-ray diffraction datasets for both are also available, under accession codes 409 and 410 at the SBGrid Data Bank (https://data.sbgrid.org).
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).	Primary diffraction data has been deposited to the SBGrid Data Bank (https://data.sbgrid.org) under accession numbers 409 and 410, as noted in the "Data Availability" section.
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	A "Data Availability" section has been included at the end of the Materials and Methods section, detailing the crystal structures reported in this manuscript and those used for data analysis/interpretation, as well as detailing the availability of primary diffraction data. All datasets are noted with a DOI.
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