

The human GID complex engages two independent modules for substrate recruitment

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(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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Matthias,

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are potentially interesting. However, the referees also point out several technical concerns and have a number of suggestions for how the study should be strengthened, and I think that all of them should be addressed. Referee 1 and 3 point out that the cryo-EM data are of rather low resolution and referee 1 suggests revisiting the processing of all EM data in C1. Other concerns relate to missing control experiments, the proposed second dimerization interface and the role of the WD40 domain of WDR26. The related manuscript from Sherpa D, Schulman B and colleagues (2021) needs to be cited and discussed.

These are all constructive comments and I feel that it is possible to address them in a reasonable timeframe. We would therefore like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We invite you to submit your manuscript within three months of a request for revision. However, we are aware of the fact that many laboratories are not fully functional due to COVID-19 related shutdowns and we have therefore extended the revision time for all research manuscripts under our scooping protection to allow for the extra time required to address essential experimental issues. Please contact us to discuss the time needed and the revisions further.

*****IMPORTANT NOTE:** we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

- 1) A data availability section is missing.
- 2) Your manuscript contains error bars based on $n=2$. Please use scatter blots showing the individual datapoints in these cases. The use of statistical tests needs to be justified.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.***

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- 1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.
 - 1a) I suggest to reformat your manuscript as a "Report" with a combined Results and Discussion section, unless the revision results in more than 5 figures. In this case these sections can remain separate.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

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<https://www.embopress.org/page/journal/14693178/authorguide> for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

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5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines

()

6) Supplementary information:

I suggest changing the supplementary figures to Expanded View figures. We can accommodate up to five of these.

The supplementary tables could be combined into a single Appendix pdf, except for Table S4 and S5. These should be uploaded as Dataset EV1 and EV2 in the form of an .xls file. If you find this option more useful, you can of course upload all 5 tables in this format.

Please find more detailed information on these file types here:

We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2' etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here:

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Data availability: Please follow the instructions below for how to format the Data availability section:

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method) that follows the model below (see also <

<https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>>). Please note

that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843
(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843>)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available .

9) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

10) Regarding data quantification

The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
- the nature of the bars and error bars (s.d., s.e.m.)

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

- Please also include scale bars in all microscopy images.

11) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

Referee #1:

Mohamed et al, present an interesting structure-function analysis of the human GID complex, a large multi-subunit E3 ubiquitin ligase complex involved in several cellular process such as glucose metabolism and cell cycle progression. The manuscript starts with siRNA-based depletion experiments for identifying critical subunits of this complex involved in substrate recruitment in HeLa Kyoto cells. This information is then used for reconstituting a recombinant version of this complex that the authors employ for performing an integrative structural biology analysis. This analysis combines cryo-EM, in vitro ubiquitination assays and cross-linking mass spectrometry. Overall the manuscript is well written and it is highly appreciable that this study involves several methods to characterise an important human E3 ligase complex, I would recommend to publish this manuscript in EMBO reports only if the authors will address my major concerns listed below with the hope that this will increase the quality of the manuscript and that this will avoid publishing some misinterpretations of the data here presented.

Major concerns:

1) The authors claim that, the GID complexes reconstituted here are D2 symmetric based on some diagnostics mentioned in the text (but not completely shown). These include: 2D class average analysis, 3D reconstruction and monitoring of 2D projections from the 3D volume (pages 17-18 lines 607-618).

Unfortunately the EM data showed is not at a resolution which would be high enough to ultimately prove that this complex is indeed D2 symmetric. It is also not recommended to apply symmetry at very early stages of the cryo-EM data processing pipeline.

Given the high resolution structure of the homologous yeast GID complex published by Sherpa et al (bioRxiv), it is obvious that this complex does not have D2 symmetry but a pseudo-C2 symmetry. Given this, the authors should process all the EM data in this manuscript in C1. The local resolution on individual modules of this complex can then be improved by 3D classification and/or focused 3D refinement procedures.

After doing this the authors should revisit the interpretation of their EM structure. The structural data from Sherpa et al, which should be cited, will be very useful in this context. At this stage it would be very interesting to compare yeast and human GID complexes.

2) The EM data should be better presented, a raw micrograph image with scale bar should be included. The scale bar is also missing in the 2D class averages shown.

Minor points:

1) Molecular markers are missing in the protein gels shown in this article, they should be included.

Referee #2:

Mohamed et al. reported Cryo-EM reconstructions of human GID complex to reveal the architecture of the complex with two different substrate-recruiting modules. Human GID complex consists of RanBP9, RMND5a, ARMC8, TWA1, MAEA, WDR26/MKLN1, GID4 and YPEL5. How these components assembled into GID complex remains unknown. Mohamed et al. showed that WDR26 and GID4 function as substrate-binding modules where WDR26 selectively binds HBP1 substrate and GID4 binds ZMYND19 substrate to promote their ubiquitination and degradation. To gain insight into the GID complex, the authors presented Cryo-EM reconstructions of complexes consisting of RanBP9, RMND5a, TWA1, MAEA and WDR26 in the absence and presence of ARMC8-beta. ARMC8-beta lacks C-terminal region compared to ARMC8-alpha and was unable to bind GID4. Both complexes adopt a similar RING-shaped tetramer, where two dimers are formed via the WD40 domain of WDR26. Cross-linking mass spectrometry (XL-MS) analysis and homology models of each component generated from prior published structure of a monomeric yeast GID complex lacking WDR26 were used to fit the model. The model suggests that WDR26 forms one dimer interface and RMND5a/MAEA form the second dimerization interface to assemble into a tetramer. ARMC8-beta does not alter the tetrameric RING-shaped structure but functions to stabilize the tetramer. A model of yeast GID4-ARMC8 was fitted into the Cryo-EM map showing that GID4 binding site does not overlap with WDR26 and hence GID complex may engage both substrate-recruiting modules simultaneously.

Overall the data are solid. The tetrameric architecture of GID complex with dual substrate-recruiting modules is intriguing and interesting.

I have few comments.

1. The authors proposed that RMND5a/MAEA form the second dimerization interface but the structural model of this complex was not fitted in the Cryo-EM map. They suggest that four RING modules could be located at this dimerization interface. Figure 5C and 5D seemingly suggest that the dotted density indicated for RMND5a/MAEA might only be sufficient to fit either RMND5a or MAEA based on yGID9. Could the authors provide evidence to verify whether one or two RMND5a/MAEA complex is located at the dimerization interface? Alternatively does RMND5a/MAEA complex form oligomer?
2. Figure 2C showed that RanBP9 has subtle effect on HBP1 ubiquitination, but the model in Figure 5 showed that RanBP9 is essential to connect WDR26 to the rest of complex. Could the authors explain the subtle effect?
3. It is unclear whether GID4 substrate recruitment and ubiquitination is dependent on WDR26-mediated tetrameric arrangement. Cell-based data in Figure 1C showed that ectopic expression of WDR26 did not affect ZMYND19 stability. Could this be tested in vitro using TWA1, RMND5a, MAEA, ARMC8 and GID4 complex?
4. Figure 1A, GID4 blot is missing.
5. Figure 1D, it is not clear which data in Figure 1 supported that ARMC8 functions with GID4 to regulate ZMYND19. Does ARMC8 affect ZMYND19 stability?

Minor comments

Fig. 2A RMMND5a typo

Fig. 3D +/- labels for HSS-WDR26deltaWD40 need correction

Line 76 ARMC88 typo

Referee #3:

A potentially interesting paper showing the roles of accessory subunits (WDR26, RanBP9 and GID4 - ARM8) in conferring specificity on hGID for its substrates HBP1 and ZMYND19, how these assemble into distinct complexes and some low resolution cryo-EM reconstructions. There is some overlap with the recent publication from Brenda Schulman on the budding yeast system (Sherpa et al., Mol Cell). However the study by Mohamed and colleagues is on human, although the quality and resolution of the cryo-EM reconstructions in this manuscript are substantially lower than in Sherpa et al. Reference needs to be made to Sherpa et al. There are a number of issues that require addressing before this paper can be accepted for publication.

1. Fig. 2 lacks controls as follows:

a. Fig. 2C, ubiquitination assay: A control assay of MAEA, RMND5a, Twa1 (Core) alone is not shown. Without this it is not possible to determine the effects of the accessory subunits on stimulating and/or repressing GIN-mediated substrate ubiquitination.

b. Mol weights should be shown in Figs 2C-E.

c. The complexes used in the assays in Fig. 2C-E are not all shown in Fig. 2a. Specifically, complexes with GID4, i.e. core-A-G and core-A-G-R-W (Figs 2D and 2E). Thus, we do not know if GID4 is actually bound in these complexes, and therefore the roles of GID4 and ARM8C in mediating HBP1 ubiquitination are inconclusive.

d. Mention that GID4 binding to the core requires ARMC8 (discussed later) could be mentioned at this point.

2. Related to point 1c, in Fig. 2, it was unclear whether GID4 alone binds the core complex. Data showing both that a core-GID4 complex can be assembled and its activity towards HBP1 and ZMYND19 need to be included.

3. The presence of the E2 should be included in Fig. 2C-E.

4. Line 201-202. The subunit composition of the two SEC peaks referred to isn't shown.

5. Line 200. What is the expected mass of the complex relative to the 1.1 MDa determined by SEC-MALS?

6. Cryo-EM reconstruction is of low resolution. The term 'subnanometer', line 230 is a little misleading. In the 2D class averages shown in Supp Fig. 2B, there are 8 protrusions into the centre of the ring-like structure. These do not seem to be reproduced in the 3D reconstructions (Fig. 3C). Can the authors comment on this.

7. Role of WD40 domain of WDR26 (lines 232-242). The suggestion that the WD40 domain of WDR26 recognises substrate is based on WD40 domain deletion in vivo (Fig. 3D). However, since this could be due to other reasons, eg complex instability, the authors should test this in vitro using their ubiquitination assay.

8. Line 250. ARM8C should be ARM8Calpha.

9. Fig. 4A. 'ID4' should be 'GID4'. What is 'Probeller'?

10. Fig. 4C. The background for ARM8Cbeta is high for the GID4 interaction experiment. It is difficult to conclude anything regarding GID4 interaction.

11. Fig. 4D. Why is GID4 present with ARM8Cbeta in the right lane if GID4 and ARM8Cbeta are not present in the same complex (line 264)? Is there a His tag on another GID subunit other than GID4?

12. Line 304: Has the suggestion that hGID can bind WDR26 and GID4 been tested?

Point-by-point letter highlighting our response to all comments/suggestions:

As outlined in the cover letter, we have thoroughly addressed all comments/suggestions and included new experiments to further substantiate specific points. We would like to thank all reviewers for their encouragement and constructive input.

Referee #1:

Mohamed et al, present an interesting structure-function analysis of the human GID complex, a large multi-subunit E3 ubiquitin ligase complex involved in several cellular process such as glucose metabolism and cell cycle progression. The manuscript starts with siRNA-based depletion experiments for identifying critical subunits of this complex involved in substrate recruitment in HeLa Kyoto cells. This information is then used for reconstituting a recombinant version of this complex that the authors employ for performing an integrative structural biology analysis. This analysis combines cryo-EM, in vitro ubiquitination assays and cross-linking mass spectrometry. Overall the manuscript is well written and it is highly appreciable that this study involves several methods to characterise an important human E3 ligase complex, I would recommend to publish this manuscript in EMBO reports only if the authors will address my major concerns listed below with the hope that this will increase the quality of the manuscript and that this will avoid publishing some misinterpretations of the data here presented.

We thank the reviewer for the valuable criticism. We would like to clarify the different strategies employed by us and the recent publication by the Schulman group. The publication of the Schulman lab (Sherpa *et al*, 2021) - in addition to the yeast GID complex - also investigated the homologous human GID complex, called CTLH, which has a similar composition as the complex studied here. Sherpa *et al*. (2021) includes the C2 symmetric map of a ring-shaped tetrameric assembly of the CTLH-SR4 complex (containing the subunits WDR26, RANBP9, GID4, ARMC8, TWA1, MAEA and RMND5A) at low resolution (19.2Å) as well as a near-atomic resolution reconstruction of a subcomplex of CTLH (containing the subunits RANBP9, ARMC8, TWA1, MAEA and RMND5A) that does not tetramerize. Due to the flexibility of the assembled ring-shaped tetramer, which is also evident in the 2D class averages, our reconstruction of the entire ring-shaped 5-subunit hGID complex (WDR26, RANBP9, TWA1, MAEA and RMND5A) was limited to a resolution of around 11.2 Å (Figure EV 2A). Sherpa *et al*. (2021) resolved the structure of a monomeric subcomplex at near-atomic resolution by omitting WDR26, which mediates formation of the

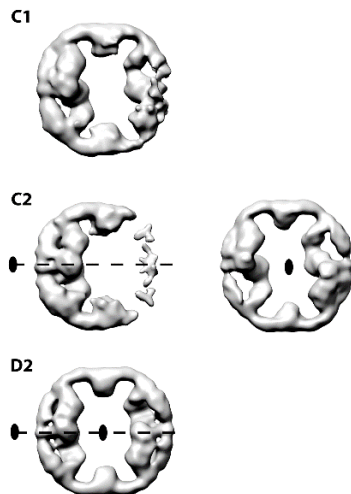
ring-shaped tetramer. We addressed the flexibility issue computationally by symmetry expansion followed by refinement of the tetrameric building block without application of symmetry. The obtained map was of sufficient resolution to position homology models of the subunits RANBP9, TWA1, and WDR26, and our model is in agreement with the high-resolution model of the sub-complex published by the Schulman group. We have improved the description of the cryo-EM analysis in the revised Result section and Supplementary information, and also expanded the Material and Method section. Further details of how we addressed the specific points related to the cryo-EM analysis are provided below.

Major concerns:

1) The authors claim that, the GID complexes reconstituted here are D2 symmetric based on some diagnostics mentioned in the text (but not completely shown). These include: 2D class average analysis, 3D reconstruction and monitoring of 2D projections from the 3D volume (pages 17-18 lines 607-618).

Unfortunately the EM data showed is not at a resolution which would be high enough to ultimately prove that this complex is indeed D2 symmetric. It is also not recommended to apply symmetry at very early stages of the cryo-EM data processing pipeline. Given the high resolution structure of the homologous yeast GID complex published by Sherpa et al (bioRxiv), it is obvious that this complex does not have D2 symmetry but a pseudo-C2 symmetry. Given this, the authors should process all the EM data in this manuscript in C1. The local resolution on individual modules of this complex can then be improved by 3D classification and/or focused 3D refinement procedures.

We calculated the initial models using no symmetry, as well as C2 and D2 symmetry and found the same structural features with all symmetry settings (illustrated in Figure below).



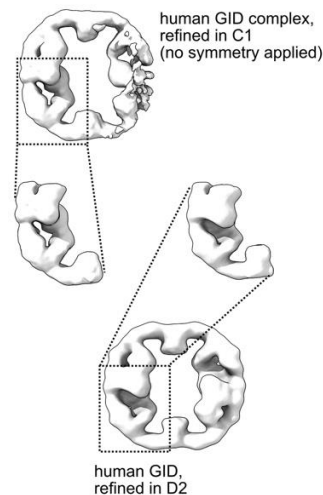
One of the C2 symmetric initial models showed symmetry around an axis perpendicular to the C2 symmetry axis suggesting a (pseudo-) D2 symmetry at the resolution reached (around 25 Å). As the reviewer correctly points out, the higher resolution structure of the Schulman group revealed that RMND5A and MAEA are present as only two copies each in the complex investigated, which renders the complex C2 symmetric rather than D2 symmetric. We thus changed the corresponding statement in the manuscript as follows to reflect this fact: «Initial model generation with CryoSPARC (Punjani *et al*, 2017) suggested a pseudo-D2 symmetric arrangement, consistent with a tetrameric assembly of the 5-subunit GID complex.»

Given this, the authors should process all the EM data in this manuscript in C1. The local resolution on individual modules of this complex can then be improved by 3D classification and/or focused 3D refinement procedures.

Our paper contains structural information on two distinct complexes, the 5-subunit hGID complex (RanBP9, WDR26, MAEA, RMND5a and TWA1) and 6-subunit hGID complex (RanBP9, WDR26, MAEA, RMND5a, TWA1 and ARMC8β), and we followed two different processing strategies to resolve their cryo-EM structures:

1) Using symmetry expansion, we processed the data by focusing the alignment on one tetrameric unit of the 5-subunit hGID complex to obtain a reconstruction with a resolution high enough to allow fitting of homology models. We would like to point out that we performed the initial classification steps without symmetry application (Figure EV 2A) and applied D2 symmetry only later directly before the symmetry expansion step. After symmetry expansion we classified and refined the tetrameric building block – consisting of WDR26, RanBP9, TWA1, and MAEA or RMND5A – again without application of symmetry. To further

corroborate that no artifacts were introduced by this processing strategy, we refined the structure of the ring-shaped hGID complex in C1. We started with an initial model obtained without symmetry applied. The best resolved part of this reconstruction refined in C1 is highly similar to the map of the tetrameric building block obtained after symmetry expansion, though it refines only to lower resolution. This is illustrated in the image below, in which a region corresponding to the tetrameric building block reconstruction was extracted from the C1 reconstruction, and the reconstructions were filtered to the same resolution.



2) To localize ARMC8 β , we processed hGID and hGID-ARMC8 β data in parallel (full processing scheme in Figure EV 3E). We reprocessed the data applying C2 symmetry, which resulted in reconstructions that are overall very similar to reconstructions obtained with D2 symmetry in the previous version of the manuscript. We replaced the maps of hGID and hGID-ARMC8 β shown in Figures 4I, J and 5A with the new maps processed with C2 symmetry in the final refinement step.

After doing this the authors should revisit the interpretation of their EM structure. The structural data from Sherpa et al, which should be cited, will be very useful in this context. At this stage it would be very interesting to compare yeast and human GID complexes.

As requested, we added citations for the Sherpa *et al.* (2021) paper, which was not published at the time we submitted our manuscript. Sherpa *et al.* (2021) show a detailed comparison of the yeast and human GID complexes (e.g. in Figure S6), and we thus did not repeat this analysis for the revisions. Our results regarding the position of the WDR26, TWA1, Armc8 β and RanBP9 subunits are in perfect agreement with the structural model shown in Sherpa *et al.* (2021). We did not fit the MAEA and RMND5A RING-containing

subunits due to the low resolution in this region. Importantly, instead of ARMC8 α , we functionally characterized and structurally mapped the naturally occurring ARMC8 β isoform, which lacks the C-terminal half. Interestingly, ARMC8 β forms a stable hGID complex with a functional RanBP9/WDR26 module, but fails to recruit GID4 (Figure 4D-F), as the C-terminus of ARMC8 α mediates a critical contact with the GID4 subunit. We have now expanded these findings and included a new experiment demonstrating that *in vitro* the GID4-module of the ARMC8 β -containing hGID complex is functionally inactive as expected (Figure 4E), while bound WDR26/RanBP9 is able to ubiquitinate HBP1 (revised Figure 4, new panel F). Together with the structural analysis reported in Sherpa *et al.* (2021), our data thus mechanistically explain the functional difference between ARMC8 α - and ARMC8 β -containing hGID complexes.

2) The EM data should be better presented, a raw micrograph image with scale bar should be included. The scale bar is also missing in the 2D class averages shown.

We have corrected this oversight by adding the requested raw micrograph panels (revised Figure EV 2A). Moreover, we added scale bars to the Figure EV panels 2B and 3F.

Minor points:

1) Molecular markers are missing in the protein gels shown in this article, they should be included.

As suggested, we have added molecular markers to all protein gels.

Referee #2:

Mohamed et al. reported Cryo-EM reconstructions of human GID complex to reveal the architecture of the complex with two different substrate-recruiting modules. Human GID complex consists of RanBP9, RMND5a, ARMC8, TWA1, MAEA, WDR26/MKLN1, GID4 and YPEL5. How these components assembled into GID complex remains unknown. Mohamed et al. showed that WDR26 and GID4 function as substrate-binding modules where WDR26 selectively binds HBP1 substrate and GID4 binds ZMYND19 substrate to promote their ubiquitination and degradation. To gain insight into the GID complex, the authors presented Cryo-EM reconstructions of complexes consisting of RanBP9, RMND5a, TWA1, MAEA and WDR26 in the absence

and presence of ARMC8-beta. ARMC8-beta lacks C-terminal region compared to ARMC8-alpha and was unable to bind GID4. Both complexes adopt a similar RING-shaped tetramer, where two dimers are formed via the WD40 domain of WDR26. Cross-linking mass spectrometry (XL-MS) analysis and homology models of each component generated from prior published structure of a monomeric yeast GID complex lacking WDR26 were used to fit the model. The model suggests that WDR26 forms one dimer interface and RMND5a/MAEA form the second dimerization interface to assemble into a tetramer. ARMC8-beta does not alter the tetrameric RING-shaped structure but functions to stabilize the tetramer. A model of yeast GID4-ARMC8 was fitted into the Cryo-EM map showing that GID4 binding site does not overlap with WDR26 and hence GID complex may engage both substrate-recruiting modules simultaneously.

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I have few comments.

1. The authors proposed that RMND5a/MAEA form the second dimerization interface but the structural model of this complex was not fitted in the Cryo-EM map. They suggest that four RING modules could be located at this dimerization interface. Figure 5C and 5D seemingly suggest that the dotted density indicated for RMND5a/MAEA might only be sufficient to fit either RMND5a or MAEA based on yGID9. Could the authors provide evidence to verify whether one or two RMND5a/MAEA complex is located at the dimerization interface? Alternatively does RMND5a/MAEA complex form oligomer?

Our map of the tetrameric building block after symmetry expansion did not reach a resolution that allowed us to reliably interpret the density corresponding to the MAEA and RMND5a subunits. We thus only indicated the position of these RING-subunits with a dotted line. Although Sherpa *et al.* (2021) determined structural maps of a slightly different hGID complex, the arrangement of the two tetrameric assemblies of four building blocks consisting of WDR26, RanBP9, TWA1, and MAEA or RMND5A, seems identical. We thus revised the text accordingly, and refer to the Sherpa *et al.* (2021) structure to further discuss the position and stoichiometry of MAEA and RMND5a.

2. Figure 2C showed that RanBP9 has subtle effect on HBP1 ubiquitination, but the

model in Figure 5 showed that RanBP9 is essential to connect WDR26 to the rest of complex. Could the authors explain the subtle effect?

The structural model presented in Figure 5 indeed shows that RanBP9 mediates extensive interactions with the WD40 domain of WDR26. Nevertheless, at least *in vitro*, RanBP9 is not essential for WDR26 recruitment to the hGID complex. This is evident by the ability of WDR26 to form a stable complex with the core subunits (MAEA, RMND5a, TWA1) in the absence of RanBP9 (Figure 2A). To corroborate these data, we now included a new experiment (revised Figure EV 4, new panel B), showing that WDR26 directly binds TWA1 *in vitro*.

Despite these distinct binding interfaces, available evidence suggest that RanBP9 fulfills a scaffolding function, explaining increased catalytic activity of RanBP9-containing hGID complexes. Indeed, size exclusion chromatography profiles of different hGid complexes confirm a stabilizing role of RanBP9 (Figure EV 1A and C). Moreover, *in vivo* experiments presented in Figure 1A demonstrate that siRNA knockdown of either RanBP9 or WDR26 lead to accumulation of HBP1, consistent with the notion that the presence of RanBP9 improves catalytic activity of the hGID complex.

3. It is unclear whether GID4 substrate recruitment and ubiquitination is dependent on WDR26-mediated tetrameric arrangement. Cell-based data in Figure 1C showed that ectopic expression of WDR26 did not affect ZMYND19 stability. Could this be tested in vitro using TWA1, RMND5a, MAEA, ARMC8 and GID4 complex?

We now added new data showing that the WD40 domain of WDR26 is not only required for HBP1 degradation *in vivo* (Figure 3D), but also for HBP1 ubiquitination *in vitro* (Figure 3, new panel E). However, the reviewer is correct that at present we cannot rigorously distinguish whether the functional relevance of the WD40 domain lies in tetramerization or substrate recognition (or both). We are currently screening for specific separation-of-function mutants to thoroughly investigate the role of tetramerization. Previous work by the Schulman laboratory (Qaio *et al.*, 2020) identified that the yeast GID complex in absence of Gid7/WDR26 is monomeric but fully functional to degrade its substrate FBP *in vivo* and ubiquitinate FBP *in vitro*. This is further substantiated by the position of FBP in the recent cryo-EM complex of the tetrameric yeast GID complex (Sherpa *et al.*, 2021), which does not involve contacts to Gid7. However, the oligomeric state of the human GID complex without WDR26 remains to be investigated, as additional dimerization interfaces are provided by the RING subunits RMND5a and MAEA. To address these interesting possibilities, we have thus revised the text to better highlight the implications of WDR26-

mediated tetramerization in the context of the previous results obtained with the monomeric yeast GID E3 ligase.

4. Figure 1A, GID4 blot is missing.

As requested, we have now included a GID4 blot using a polyclonal antibody kindly provided by Brenda Schulmann. As shown in the revised Figure 1A, our GID4 RNAi oligos clearly reduce GID4 levels, and interestingly, GID4 is also reduced in cells RNAi-depleted for WDR26, albeit to a lesser extent.

5. Figure 1D, it is not clear which data in Figure 1 supported that ARMC8 functions with GID4 to regulate ZMYND19. Does ARMC8 affect ZMYND19 stability?

Unfortunately, our available antibody does not detect endogenous ZMYND19 protein, and we can thus not examine hGID-dependent degradation of endogenous ZMYND19 *in vivo*. However, several lines of evidence suggest that full-length ARMC8 is essential for GID4 recruitment to the hGID complex and for activity towards ZMYND19. In pull-down experiments GID4 fails to bind the hGID complex in the absence of ARMC8 (Figure EV 3A), and similar conclusions were reached based on the yeast GID structure (Qaio et al., 2020). Importantly, we now show that a stable hGID complex assembled with the shorter ARMC8 β isoform fails to bind GID4 and exhibits no GID4-dependent E3 ligase activity (Figure 4E), while it maintains activity towards HBP1 (Figure 4, new panel F). Together, these data demonstrate that the GID4-ARMC8 α module is required for activity towards ZMYND19, while the WDR26-RanBP9 module functions independently to ubiquitinate HBP1 and presumably other targets (Figure 5).

Minor comments

Fig. 2A RMMND5a typo

Thanks - we corrected this typo.

Fig. 3D +/- labels for HSS-WDR26deltaWD40 need correction

This is an important correction - thank you.

Line 76 ARMC88 typo

We adjusted this typo.

Referee #3:

A potentially interesting paper showing the roles of accessory subunits (WDR26, RanBP9 and GID4 - ARM8) in conferring specificity on hGID for its substrates HBP1 and ZMYND19, how these assemble into distinct complexes and some low resolution cryo-EM reconstructions. There is some overlap with the recent publication from Brenda Schulman on the budding yeast system (Sherpa et al., Mol Cell). However the study by Mohamed and colleagues is on human, although the quality and resolution of the cryo-EM reconstructions in this manuscript are substantially lower than in Sherpa et al. Reference needs to be made to Sherpa et al. There are a number of issues that require addressing before this paper can be accepted for publication.

1. Fig. 2 lacks controls as follows:

a. Fig. 2C, ubiquitination assay: A control assay of MAEA, RMND5a, Twa1 (Core) alone is not shown. Without this it is not possible to determine the effects of the accessory subunits on stimulating and/or repressing GID-mediated substrate ubiquitination.

We thank the reviewer for suggesting this critical control. We now performed this experiment and found that the core complex comprised of TWA1, MAEA and RMND5a fails to efficiently ubiquitinate HBP1, as compared to the core complex with RanBP9 and WDR26 (Figure EV 1, new panel H).

b. Mol weights should be shown in Figs 2C-E.

As requested, we have added the position of the molecular weight markers.

c. The complexes used in the assays in Fig. 2C-E are not all shown in Fig. 2a. Specifically, complexes with GID4, i.e. core-A-G and core-A-G-R-W (Figs 2D and 2E). Thus, we do not know if GID4 is actually bound in these complexes, and therefore the roles of GID4 and ARMC8 in mediating HBP1 ubiquitination are inconclusive.

We did not use hGID complexes assembled by co-expressing GID4, as a significant fraction of GID4 is lost during the purification steps (i.e. ion exchange and size exclusion). To circumvent heterogeneous stoichiometries of hGID complexes, we thus expressed and purified the GID4 subunit separately (Figure 2A), and added it to ubiquitination reactions. We

only collected the soluble fractions corresponding to the expected protein molecular weight from the size exclusion column. A similar approach was also followed in Sherpa *et al.* (2021), suggesting that co-expressing GID4 with the other hGID subunits may not be successful.

From *in vitro* binding experiments (Figure EV 3A), we know that GID4 is efficiently incorporated into ARMC8 α -containing hGID complexes. Moreover, purified GID4 is soluble and functional, as ZMYND19 is only ubiquitinated in the presence of GID4 (Figure 2E), and this activity can be inhibited with a peptide blocking the substrate interaction pocket of GID4. In contrast, hGID complexes assembled with ARMC8 β fail to bind GID4 and do not support GID4-dependent ubiquitination activity, while HBP1 ubiquitination through the WDR26/RanBP9 module is maintained (Figure 4, panel D-F). We conclude from these data that GID4 and ARMC8 are not required for HBP1 ubiquitination, consistent with the *in vivo* results shown in Figure 1A and 1B.

d. Mention that GID4 binding to the core requires ARMC8 (discussed later) could be mentioned at this point.

As suggested, we adjusted the text and now mention that ARMC8 is required for GID4 recruitment already in this section of the manuscript.

2. Related to point 1c, in Fig, 2, it was unclear whether GID4 alone binds the core complex. Data showing both that a core-GID4 complex can be assembled and its activity towards HBP1 and ZMYND19 need to be included.

We apologize that this point was not clear, and revised the text to better explain that ARMC8 is essential for GID4 recruitment to the hGID complex, as shown in Figure EV 3A. We further show that GID4 can only be recruited via ARMC8 α , but not the shorter ARMC8 β isoform. We now included new experiments showing that the hGID-ARMC8 β complex is unable to ubiquitinate GID4 targets, but maintains its activity towards the WDR26-RanBP9 target HBP1 (Figure 4, new panel F).

3. The presence of the E2 should be included in Fig. 2C-E.

Ubiquitin E1 and E2 were always present in these assays, as indicated in the Figure legend. The negative controls lack ubiquitin.

4. Line 201-202. The subunit composition of the two SEC peaks referred to isn't shown.

Thank you for pointing this out. We revised the corresponding text and now include the subunit composition of the analyzed hGID complexes.

5. Line 200. What is the expected mass of the complex relative to the 1.1 MDa determined by SEC-MALS?

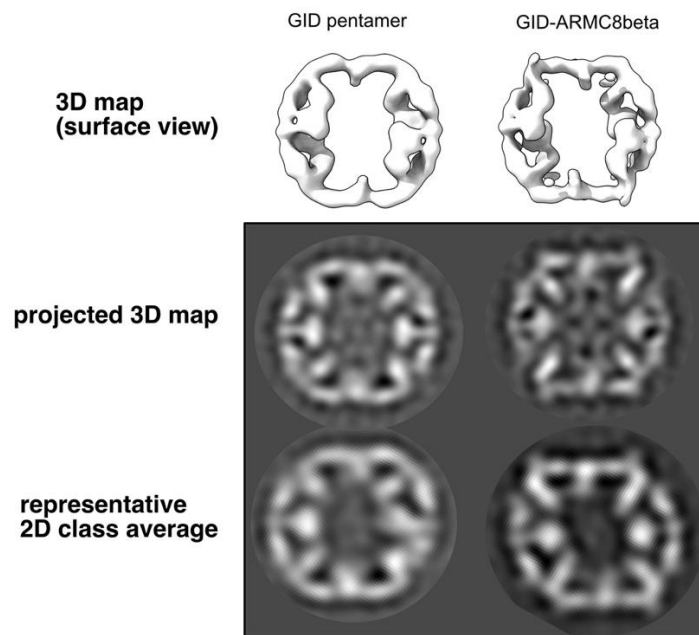
The expected molecular weight of the tetrameric complex is 1060 kDa, which is now mentioned in the revised text.

6. Cryo-EM reconstruction is of low resolution. The term 'subnanometer', line 230 is a little misleading.

We agree that the local resolution of the map is in some parts significantly lower than 10 Å and have thus changed the wording in line 239 as follows: «we employed 3D classification after symmetry expansion to refine a cryo-EM map of the tetrameric building block (RanBP9, WDR26, TWA1, MAEA or RMND5a) to higher resolution (9 Å, FSC=0.143 criterion)».

In the 2D class averages shown in Supp Fig. 2B, there are 8 protrusions into the centre of the ring-like structure. These do not seem to be reproduced in the 3D reconstructions (Fig. 3C). Can the authors comment on this.

Of the eight protrusions, two originate from MAEA-RNMD5a complexes, and four from RanBP9. Although there are four beta-propellers of WDR26 in the complex, when projected in top view, two beta-propellers overlap (see Figure 3C) and give rise to a single protrusion (see Figure below), explaining the remaining two protrusions.



7. Role of WD40 domain of WDR26 (lines 232-242). The suggestion that the WD40 domain of WDR26 recognises substrate is based on WD40 domain deletion *in vivo* (Fig. 3D). However, since this could be due to other reasons, eg complex instability, the authors should test this *in vitro* using their ubiquitination assay.

We agree with the reviewer that complex instability cannot be excluded, and we indeed noticed that the purified TWA1, MAEA, RMND5a, RanBP9, WDR26 (Δ WD40) complex was somewhat less stable upon concentration. As suggested, we now performed *in vitro* HBP1 ubiquitination assays, and as expected, we observed significantly less ubiquitination activity with the WDR26 (Δ WD40) complex compared to WDR26 wild type controls (Figure 3, new panel E).

8. Line 250. ARM8C should be ARM8Calpha.

Thanks - we have changed this.

9. Fig. 4A. 'ID4' should be 'GID4'. What is 'Probeller'?

We adjusted these errors. GID4 indeed contains a β -barrel domain not a Propeller, apologies for this mistake.

10. Fig. 4C. The background for ARMC8beta is high for the GID4 interaction experiment. It is difficult to conclude anything regarding GID4 interaction.

We agree with the reviewer that the strong background in this blot makes it difficult to draw solid conclusions about ARMC8 β binding to GID4. Therefore, we changed the set-up of our experiment, and now overexpressed HSS-tagged ARMC8 constructs (α or β) with FLAG-GID4 in HEK293T cells. In this new experiment, it is clear that FLAG-GID4 is only present in ARMC8 α - but not ARMC8 β immunoprecipitates (revised Figure 4, new panel C).

11. Fig. 4D. Why is GID4 present with ARMC8beta in the right lane if GID4 and ARMC8beta are not present in the same complex (line 264)? Is there a His tag on another GID subunit other than GID4?

Thanks for highlighting this source of misunderstanding. The right lane indeed represents a His-tag pull-down control to confirm that GID4 is expressed, but does not incorporate in the complex, as shown by pulling on ARMC8 β . We have now re-phrased the text and adjusted the Figure accordingly.

12. Line 304: Has the suggestion that hGID can bind WDR26 and GID4 been tested?

We agree with the reviewer that this is an important experiment. To address this point, we have performed immunoprecipitation experiments in HEK293T cells, demonstrating that the presence of endogenous WDR26 in hGID complexes isolated in FLAG-GID4 immunoprecipitations (Figure EV 4, new panel E). Consistent with this notion, we further show that GID4 is absent in hGID complexes containing ARMC8 β , but the complex still ubiquitinates HBP1 via the WDR26-RanBP9 module (Figure 4, new panel F). Together, these results indicate that although the ARMC8/GID4 and RanBP9/WDR26 modules function independently, both can be present in hGID tetramers.

Dear Matthias,

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, all referees are very positive about the study and request only minor changes, i.e., the inclusion of data in the Supplement.

Browsing through the manuscript myself, I noticed a few editorial things that we need before we can proceed with the official acceptance of your study.

- Your manuscript contains 5 figures and should be published in our Reports section. This requires that you combine the Results and Discussion section, please.

- Please provide up to 5 keywords.

- Funding: Please complete the information on funding in our online submission system. Currently, the grant numbers are missing.

- Please change the labels on the EV figures themselves to Figure EVx instead of "Supplementary figure X".

- Data availability section: please provide links that resolve to the deposited datasets on EMD and PRIDE.

- Figure 3D and 4C, the contrast of the blots has been enhanced quite a lot compared to the source data and should be reduced.

- Figure EV1H: It seems that you marked the wrong lanes in the source data file. Lane 1 and 2 should be next to each other. Please double-check.

- Source data for Figure EV4E: The source data for Input and FLAG-IP does not match the data in the figure panel, unless you flipped the orientation of the original blots. Please double-check and correct this.

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We look forward to seeing a final version of your manuscript as soon as possible.

With kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

Referee #1:

RE: Point-by-point letter highlighting our response to all comments/suggestions.

The authors addressed my comments and concerns, I recommend publication of the manuscript after a final last amendment to be done:

"We calculated the initial models using no symmetry, as well as C2 and D2 symmetry and found the same structural features with all symmetry settings (illustrated in Figure below)." This figure should be added in the Supplementary Figure 2.

Referee #2:

The authors have addressed all my concerns and the manuscript is suitable for publication.

Referee #3:

The authors have satisfactorily addressed my concerns and this interesting paper is now suitable for publication.

Dear Dr. Rambold,

Please find below our response to the raised comments:

- Your manuscript contains 5 figures and should be published in our Reports section. This requires that you combine the Results and Discussion section, please.

As suggested, we have now combined the Results and Discussion section, and the manuscript should now be compatible with the EMBO Reports' format.

- Please provide up to 5 keywords.

hGID/CTLH, E3 ligase, substrate-receptors, ubiquitin, oligomerization.

- Funding: Please complete the information on funding in our online submission system. Currently, the grant numbers are missing.

The relevant grant numbers supporting this work are as follows: SNSF 310030_179283/1, Swiss Cancer League KLS-4574-08-2018 and EU-ITN 860517. These numbers have now been added to the main text and on your system.

- Please change the labels on the EV figures themselves to Figure EVx instead of "Supplementary figure X".

We changed the labels of the Supplementary Figures, as suggested.

- Data availability section: please provide links that resolve to the deposited datasets on EMD and PRIDE.

We have made the PRIDE dataset public, and here is the link that resolves to the data set:
<https://www.ebi.ac.uk/pride/archive/projects/PXD024822>

The EMDB datasets will be released when we have the DOI, so that it can be linked to the publication. The links to the datasets would be as follows:

<https://www.ebi.ac.uk/emdb/entry/EMD-13206>
<https://www.ebi.ac.uk/emdb/entry/EMD-13207>
<https://www.ebi.ac.uk/emdb/entry/EMD-13209>
<https://www.ebi.ac.uk/emdb/entry/EMD-13210>

These links have now been incorporated in the Data availability section in the revised Manuscript.

- Figure 3D and 4C, the contrast of the blots has been enhanced quite a lot compared to the source data and should be reduced.

We have adjusted the contrast in Figures 3D and 4C, as suggested.

- Figure EV1H: It seems that you marked the wrong lanes in the source data file. Lane 1 and 2 should be next to each other. Please double-check.

You are right - thanks for spotting this mistake. We have adjusted Figure EV1H accordingly.

- Source data for Figure EV4E: The source data for Input and FLAG-IP does not match

the data in the figure panel, unless you flipped the orientation of the original blots. Please double-check and correct this.

Thank you for pointing this out. The source data Figure is in the correct orientation, but the data figure was mistakenly flipped. We have now fixed this.

- I attach to this email a related manuscript file with comments by our data editors. Please address all comments and upload a revised file with tracked changes with your final manuscript submission. Please also shorten the abstract to 175 words.

We have already addressed all the comments from the data editor before submitting the revised manuscript, except for the links to datasets (see above). The PRIDE dataset is now published, however, please note that the EMDB datasets will only be released when we have a DOI, so that EMDB can link it to the publication. The abstract contains 175 words, as requested.

- Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-600 pixels large (width x height) in .png format. You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

The following information should be added to the on-line version of the manuscript.

A)

We discovered that the human GID E3 ligase complex forms tetramers with two distinct substrate-recruitment modules, namely WDR26-RanBP9 and GID4-ARMC8 α . Although the shorter ARMC8 β isoform stably assembles into the hGID complex, it lacks the ability to recruit the GID4 substrate-receptor.

B)

- The hGID complex recruits two substrate-recruitment modules, namely WDR26-RanBP9 and GID4-ARMC8 α , which in turn target distinct substrates.
- The hGID complex assembles oligomeric complexes via the RING subunits and WDR26 as interfaces, respectively.
- The hGID complex stably recruits a specific isoform of the ARMC8 subunit, namely ARMC8 β , which lacks a binding site for the GID4 substrate-receptor.

Point-by-point letter highlighting our response to the remaining comments/suggestions raised by the reviewers.

Referee #1:

The authors addressed my comments and concerns, I recommend publication of the manuscript after a final last amendment to be done:

"We calculated the initial models using no symmetry, as well as C2 and D2 symmetry and found the same structural features with all symmetry settings (illustrated in Figure below). " This figure should be added in the Supplementary Figure 2.

As suggested, we now incorporated this Figure panel into our processing scheme shown in Figure EV2.

Referee #2:

The authors have addressed all my concerns and the manuscript is suitable for publication.

Referee #3:

The authors have satisfactorily addressed my concerns and this interesting paper is now suitable for publication.

Thank you for your feedback,

Best regards,

Weaam Mohamed and Matthias Peter

Prof. Matthias Peter
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Institute of Biochemistry
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Zurich, Zurich 8093
Switzerland

Dear Matthias,

Thank you for submitting your revised manuscript. I am now very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Corresponding Author Name: Matthias Peter

Journal Submitted to: EMBO Reports

Manuscript Number: EMBOR-2021-52981V1

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.
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- 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 χ^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?	Not applicable
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Not applicable
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not applicable
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.	Not applicable
For animal studies, include a statement about randomization even if no randomization was used.	Not applicable
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Is the variance similar between the groups that are being statistically compared?	Not applicable

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), IDegreeBio (see link list at top right).	These citations/catalog numbers were provided in the Materials and methods section of the revised manuscript.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	

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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.	Not applicable
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Not applicable
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.	Not applicable

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Not applicable
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.	Not applicable
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15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable
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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	This is now included in our revised Manuscript.
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).	
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).	
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 Biocompare (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.	

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.	Not applicable
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