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Peer Review File

Cereblon induces G3BP2 neosubstrate degradation using molecular surface mimicry

Corresponding Author: Dr Georg Petzold

Version 0:

Decision Letter:

19th May 2025

Dear Dr. Petzold,

Thank you again for submitting your manuscript "Molecular surface mimicry enables CRBN to target G3BP2 for degradation". I apologize for the delay in sharing our decision. I am writing to let you know that we have decided to send your manuscript for peer review, but several points require your attention before we can proceed with peer review. Additionally we note that you have only provided names for excluded reviewers; if you would like to provide names for suggested reviewers, please do so in an updated cover letter.

I am re-opening the manuscript submission system for you to resubmit your manuscript with all associated files needed for peer review directly, within 2-3 business days if possible. Please follow the link at the bottom of this email to upload the documents listed below. If you have any issues, please reach out to us before completing the submission.

1- We require official wwPDB validation reports for newly described atomic structures, as per journal policy. We also request that authors provide cryo-EM maps, half-maps and models, as well as maps and models obtained from subtomogram averaging if it applies to the work, to help the reviewers in assessing the work. We recommend the use of figshare in our system, which allows for provision of anonymous access links for the referees (https://www.springernature.com/gp/authors/research-data/figshare-integration). Alternatively, please upload .zip folders directly with the submission. To ensure the ease of reviewer access to the data, please specify in the Data Availability section where the files can be found (i.e., provide a figshare link or direct the reader to the manuscript files).

2- We want to ensure that the methods and statistics reporting in our papers are of the highest quality. To that end, we ask authors to fill out a Reporting Summary that collects information on experimental design and reagents, as well as an editorial Policy Checklist, which confirms compliance with our editorial policies, including the declaration of Competing Interests. If your paper includes ChIP-seq, flow cytometry or MRI data, we ask you take special care to complete those sections of the Reporting Summary as this data will aid greatly in the review of your manuscript. These documents can be found by following the links below:

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3- In order for us to proceed with peer review, if relevant, please provide accession numbers and reviewer tokens to access sequencing or proteomics datasets if any unpublished datasets are part of your study. Please add this information to your manuscript file.

4- Lastly, if relevant, I would like to kindly request that you provide the code used to analyse the data to the reviewers, if newly developed (unpublished) code was used in the work. For the reviewers to evaluate the work adequately, they must be able to test the software/review the code themselves. If you have not yet provided the software, we therefore request that you provide a single compressed zip file containing the software with a readme.txt file or other user manual containing complete instructions for installing and running the software. If appropriate, please provide example data and expected output. Sufficient material should be provided for referees to directly test the performance of the software/algorithm. If the software and materials are small enough to fit in a single compressed zip file under 6MB in size, you may email this file directly to me. If the zip file is between 6 MB and 200 MB, you may upload it to our file transfer site. If necessary, a second zip file up to 200

MB in size can be used to supply the example data. Please let me know if you need to use this option and I'll send you further details. Alternatively, you can upload the code to GitHub and provide us with the link.

Please fill out and return to me the code and software submission checklist that will be made available to editors and reviewers during manuscript assessment. Please note that this form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader, instead of opening it in a web browser.
https://www.nature.com/documents/nr-software-policy.pdf

Please use the link below to submit the files. **Please also remember to move forward all other files associated with this version of the paper.**

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Dimitris Typas
Senior Editor
Nature Structural & Molecular Biology
ORCID: 0000-0002-8737-1319

Version 1:

Decision Letter:

24th Jun 2025

Dear Dr. Petzold,

Thank you again for submitting your manuscript "Molecular surface mimicry enables CRBN to target G3BP2 for degradation". We now have comments (below) from the 3 reviewers who evaluated your paper. Please note that Reviewers #2 and #3 reviewed the manuscript together, hence the identical report. In light of this feedback, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that though all referees appreciate the potential of the study, they raise important concerns that undermine the strength of the conclusions. These concerns must be fully addressed in a revised manuscript. In particular, Reviewer #1 raises key technical issues with respect to the CRBN F150 and I152V mutants used and whether they simply weaken the interaction interface with the MGD, as well as whether the ligand is fully seen bound with sufficient resolution to be convincing. Furthermore, Reviewers #2 and #3 request a quantitative evaluation of potency of the molecular interactions and degradation, with relevant guidance that we request that you follow. In addition to these points, the experts ask for expansion of the introduction and discussion, important clarifications, improved and more intuitive structural presentation, and maybe most importantly toning down claims about generalisability of the findings which they deem, and we editorially agree, as preliminary and not currently sufficiently supported. Finally, Reviewer #1 raises potential novelty concerns with respect to the notion that G-loop independent interactions of CRBN have already been inferred. We ask you to please ensure that the novelty of the work is sufficiently highlighted, showcased, and exemplified so that any similar concerns by readers or this reviewer are alleviated.

Please be sure to address/respond to all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file. If you have comments that are intended for editors only, please include those in a separate cover letter.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We expect to see your revised manuscript within 3-4 months. If you cannot send it within this time, please contact us to discuss an extension; we would still consider your revision, provided that no similar work has been accepted for publication at NSMB or published elsewhere.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf

Please note that the form is a dynamic ‘smart pdf’ and must therefore be downloaded and completed in Adobe Reader.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-portfolio/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures. Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. While these data can be displayed in a relatively informal style, they must refer back to the relevant figures. These data should be submitted as source data with the last revision, prior to acceptance.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.
-- For any revision that includes light microscopy data, we ask our authors to please include a completed light microscopy reporting table [https://www.nature.com/documents/Light_microscopy_reporting_table.xlsx] to ensure the methods are described thoroughly. The table will be available to reviewers and ultimately published should the manuscript be accepted at the journal.

EXTENDED DATA FIGURES

When re-submitting your manuscript, please ensure that any supplementary figures and tables that are crucial to the manuscript’s conclusions are converted into Extended Data figures and tables to increase visibility of these data. Extended Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

Data availability: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. Deposition of deep sequencing and microarray data is mandatory, and the datasets must be released prior to or upon publication. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at http://www.nature.com/nsmb/authors/submit/index.html#costs

Nature Structural & Molecular Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as ‘corresponding author’ on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on

‘Modify my Springer Nature account’. For more information please visit please visit www.springernature.com/orcid.

Please use the link below to submit your revised manuscript and related files:

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Dimitris Typas
Senior Editor
Nature Structural & Molecular Biology
ORCID: 0000-0002-8737-1319

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

This manuscript by Annunziato et al., shows that a molecular glue degrader (MGD) named MRT_5702 enables CRBN-mediated degradation of G3BP2. Authors also determined the cryo-EM structure of the trimeric complex between CRBN, MRT-5702 and the NTF2L homodimeric domain of G3BP2. G3BP2 does not contain G-loop motif which is typically found in CRBN neosubstrates. In their previous work (preprint, reference 3), they did show that CRBN engages with neosubstrates without G-loop for eg. VAV1. Although the data presented in this manuscript is of high quality, some of the main conclusions drawn are exaggerated or better supported with more careful generation of structure figures. Major specific comments are below.

1) Figure 4 legend reads “G3BP2 recruitment bypasses the canonical neosubstrate binding site on CRBN”. This seems like an exaggeration. From the structure shown, it appears that G3BP2 in fact is primarily engaged by the IMiD binding pocket in the CULT domain. Authors argue that some residues lining the IMiD binding pocket in the CULT domain are not involved in binding to G3BP2 and G3BP2 in fact interacts with the LON domain of CRBN specifically with residues F150 and I152. There are some concerns with these conclusions made by the authors based on the cryo-EM structure. See below

2) The overall resolution of the cryo-EM structure is 2.5A. From the extended data figure 3 where the local resolution is projected onto the cryo-EM map, it is evident that most of the G3BP2 and the interface with CRBN is resolved to a much lower resolution of 3.5 to 4.5A (authors do mention this) where interpreting specific interactions becomes very tricky. For eg. the the Extended figure 4i shows the density of the ligand which seems to be of low resolution. Can the authors confirm if they see the full ligand bound because in this Extended figure 4i, only a part of it seems to be visible. Figure 2F which is very important to support the main conclusion of the paper for eg. should include electron density to convince readers of the accuracy of fitting these residues at low resolution.

3) Do the residues F150 and I152 from the LON-loop also engage the glue? Can the authors provide data to show their involvement (or the lack of it) in the binding of the glue? Did the authors consider the possibility that the loss of binding to G3BP2 by CRBN F150 and I152v mutants is perhaps due to the weakened glue binding to CRBN in general. Then the role of LON domain is only passive in the interaction between CRBN and G3BP2 as opposed to active role that is currently claimed.

4) Authors also present a comparison of CRBN-G3BP2 structure with other structures in Figure 4. One of the structures shown is the complex of CRBN in complex with VAV1. Can the authors please clarify if this is a model generated or a cryo-EM structure? If it is already published, it would help the manuscript if the authors can discuss the novel aspects of CRBN-G3BP2 structure in comparison to CRBN-VAV1 complex structure which also lacks G-loop and which also engages the LON-loop in CRBN.

5) One of the last sentences in the manuscript reads “The rationalization of the novel G3BP2 binding mode suggests that MGD-induced protein contacts between CRBN and its neosubstrates rely on at least three determinants: (i) a preexisting PPI hotspot on either side of the interface, (ii) partial molecular mimicry of an endogenous PPI and (iii) complementation of these contacts by MGD-induced neointeractions.” Authors present one structure of CRBN in complex with G3BP2, this alone is not enough to derive general conclusions about the CRBN binding to neosubstrates. Moreover the 3 points mentioned here are general principles that can be derived from many of the previous CRBN-glue-neosubstrate complex structures including the ground-breaking first structures with IMids.

Overall, I am fairly enthusiastic about the manuscript but the authors need to tone down some of the claims about the novelty and implications of the structure. Authors should also present their structure figures especially where the ligand and the surrounding areas are shown with the electron density clearly visible and in two different views.

Reviewer #2 (Remarks to the Author):

The manuscript by Annunziato et al. describes the characterisation of a novel recognition interface between CRBN and G3BP2 in the presence of a molecular glue degrader. Following an initial observation of potent and selective degradation of G3BP2 in a global proteomics experiment, the authors obtained genetic and biochemical evidence demonstrating that compound MRT-5702 induced formation of a ternary complex with CRBN and the neosubstrate. Protein domain mapping was used to determine that the NTF2L domain of G3BP2 was necessary and sufficient for this interaction to occur.
Since initial computational analyses of G3BP2 based on known CRBN degrons were unable to rationalise the binding, the authors hypothesised that CRBN could instead be mimicking a recognition motif for G3BP2. Structural similarities between the LON domain of CRBN and USP10 (a known interactor of G3BP2) were identified in computational modelling, suggesting the formation of a novel PPI at a pre-existing hotspot in a G-loop independent manner. This was supported experimentally with NanoBRET constructs, which indicated that mutations in the alternative binding regions reduced or abolished ternary complex formation. A cryo-EM structure of the complete ternary complex was obtained, and validated the predicted interface between CRBN, G3BP2 and MRT-5702. The four diastereomers of the MGD were isolated and tested separately via TR-FRET of ternary complex formation, demonstrating clear stereoselectivity of the interaction for a particular isomer, which was assigned based on crystallographic and computational data.

Overall, the study presents a clear and concise investigation into an interesting and novel interaction. It is clearly written and well presented, and the hypotheses and computational predictions are sufficiently supported by experimental evidence. Given the broad interest in targeted protein degradation as a therapeutic modality, this study will be of great interest as it suggests a new protein interface on CRBN that can be accessed by molecular glues to mediate recognition of neosubstrates.
There are a number of points the authors should address to make the manuscript more easily accessible to the reader, which are listed below.

Major points
• Much more information is required in the introduction to provide proper context for the work. The broader implications of the study are currently unclear. What is already known about the CULT and LON domains of CRBN, including outside of the context of molecular glue degraders? Have endogenous binders or functions of the LON domain been identified? Detailed background information on CRBN, including an introductory figure on the structure and function of its domains, would put the study more clearly into context for readers (could be added to Extended Data).
• The introduction should also make a clear distinction where the previous work ends (e.g. the proteome-wide map of human CRBN target space, described in line 60) and the new results begin (presumably the global proteomics experiment in which G3BP2 degradation was first observed).
• Please also introduce MRT-5702, how it was discovered, and some context for the global proteomics experiment in which G3BP2 degradation was observed.
• Line 66: The authors report that MRT-5702 “potently reduces” levels of G3BP2 as measured by global proteomics. The study is missing a quantitative evaluation of potency of the molecular interactions and degradation; these would be of significant interest in the context of this novel CRBN protein-protein interaction. For example, DC50/Dmax for the compound should be estimated experimentally (e.g. based on NanoBRET, HiBiT, western blot, or global proteomics), and biophysical techniques (e.g. SPR) could be used to measure the cooperativity (α) of ternary complex formation.

Minor points
• In the abstract, the findings are described as a “generalisable concept”. However, this manuscript describes an investigation into a single instance of a novel protein-protein interaction, so whether this is generalisable remains to be seen. Please adjust the statement.
• Figures containing protein structures could be more clearly presented:
o The contrast in the colour schemes of protein chains in structural figures should be increased in order to clearly distinguish between different domains and proteins.
o In Extended Data Fig. 2a-2c, helices α1 & α2 have been misrendered as bent cylinders. This should be corrected for clarity and consistency with other figures.
o The use of spheres to show specific amino acids or structural features is not very helpful. Ball and stick representation might be clearer to illustrate the importance of specific residues/contacts/elements.
• Line 118: “deployed” doesn’t make sense, should this be “recognised”, “exploited”, “bound”?
• Line 135: “Ablated” is an overstatement here - F150A mutation reduces binding of full-length wtG3BP2 but it is not completely lost, “reduced” would be more appropriate.
• In the supplementary methods, there is an error in the procedure for the final step in the synthesis of MRT-5702: the mass of the amine coupled to intermediate 8 does not match the moles/equivalents reported. The other steps of the synthesis should be proofread to check for similar errors.

Reviewer #3 (Remarks to the Author):

“Molecular surface mimicry enables CRBN to target G3BP2 for degradation”
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Version 2:

Decision Letter:

Our ref: NSMB-A51030B

27th Oct 2025

Dear Dr. Petzold,

Thank you for submitting your revised manuscript "Molecular surface mimicry enables CRBN to target G3BP2 for degradation" (NSMB-A51030B). It has now been seen by the original referees and their comments are below. Please note that like in the initial round Reviewer #2 and #3 co-reviewed the manuscript. The reviewers find that the paper has improved in revision, and therefore are happy to accept the manuscript in principle in Nature Structural & Molecular Biology, pending revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about 2-3 weeks. Please do not upload the final materials and make any revisions until you receive this additional information from us.

To facilitate our work at this stage, it is important that we have a copy of the main text as a word file. If you could please send along a word version of this file as soon as possible, we would greatly appreciate it; please make sure to copy the NSMB account (cc'ed above).

Thank you again for your interest in Nature Structural & Molecular Biology Please do not hesitate to contact me if you have any questions.

Sincerely,

Dimitris Typas

Senior Editor
Nature Structural & Molecular Biology
ORCID: 0000-0002-8737-1319

Reviewer #1 (Remarks to the Author):

All my concerns have been addressed sufficiently by the authors. I recommend this manuscript be published.

Reviewer #2 + Reviewer #3 (Remarks to the Author):

To respond to the reviewers' concerns raised, the authors have made extensive changes to the manuscript including incorporation of new experiments, changes to visualization of data as well as providing additional context to the study. This has significantly improved the clarity of the manuscript and its overall message. All our concerns have been addressed, and we now fully support publication of this work.

Version 3:

Decision Letter:

12th Dec 2025

Dear Dr. Petzold,

We are now happy to accept your revised paper "Cereblon induces G3BP2 neosubstrate degradation using molecular surface mimicry" for publication as an Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Sincerely,

Dimitris Typas
Senior Editor
Nature Structural & Molecular Biology
ORCID: 0000-0002-8737-1319

Response to Editor and Reviewer comments

Editor comments:

Thank you again for submitting your manuscript "Molecular surface mimicry enables CRBN to target G3BP2 for degradation". We now have comments (below) from the 3 Reviewers who evaluated your paper. Please note that Reviewers #2 and #3 reviewed the manuscript together, hence the identical report. In light of this feedback, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that though all referees appreciate the potential of the study, they raise important concerns that undermine the strength of the conclusions. These concerns must be fully addressed in a revised manuscript. In particular, Reviewer #1 raises key technical issues with respect to the CRBN F150 and I152V mutants used and whether they simply weaken the interaction interface with the MGD, as well as whether the ligand is fully seen bound with sufficient resolution to be convincing. Furthermore, Reviewers #2 and #3 request a quantitative evaluation of potency of the molecular interactions and degradation, with relevant guidance that we request that you follow. In addition to these points, the experts ask for expansion of the introduction and discussion, important clarifications, improved and more intuitive structural presentation, and maybe most importantly toning down claims about generalisability of the findings which they deem, and we editorially agree, as preliminary and not currently sufficiently supported. Finally, Reviewer #1 raises potential novelty concerns with respect to the notion that G-loop independent interactions of CRBN have already been inferred. We ask you to please ensure that the novelty of the work is sufficiently highlighted, showcased, and exemplified so that any similar concerns by readers or this Reviewer are alleviated.

Response: We thank all Reviewers for the careful evaluation of our manuscript and the valuable feedback that overall improved the presentation of the work and led to a higher quality manuscript. We have addressed all comments made by the Reviewers, particularly those concerning resolution and quality of the cryo-EM data, and we performed additional experiments related to binary MGD engagement by mutant CRBN and DC50/EC50 quantifications as appropriate. Also, we upgraded visualization of all structural figures with a special focus on the novelty of the G3BP2 binding mode compared to G-loop targets and G-loop surface mimics (VAV1), and we rephrased and

toned down our conclusions with regards to the generalizability of our findings. Supplementary figures have been improved to show the density around the ligand and key parts of the interface to further support our conclusions. Finally, we expanded the introduction and discussion to provide more context on prior CRBN knowledge and to discuss the impact, novelty, and broader implications of our findings in the field of molecular glues and targeted protein degradation.

Reviewer #1 (Remarks to the Author):

This manuscript by Annunziato et al., shows that a molecular glue degrader (MGD) named MRT_5702 enables CRBN-mediated degradation of G3BP2. Authors also determined the cryo-EM structure of the trimeric complex between CRBN, MRT-5702 and the NTF2L homodimeric domain of G3BP2. G3BP2 does not contain G-loop motif which is typically found in CRBN neosubstrates. In their previous work (preprint, reference 3), they did show that CRBN engages with neosubstrates without G-loop for eg. VAV1. Although the data presented in this manuscript is of high quality, some of the main conclusions drawn are exaggerated or better supported with more careful generation of structure figures.

Response: We thank the Reviewer for appreciating the high quality of our manuscript, and we a grateful for raising the concerns that structural representations may have fallen short in supporting our conclusions. We believe insufficient explanation of the concept of the structural G-loop degron and the binding mode of the G-loop surface mimic VAV1 might have been the cause for the potential confusion. In the revised manuscript we have clarified the novelty of the G3BP2 binding mode in both text and figures.

Major specific comments are below.
1) Figure 4 legend reads "G3BP2 recruitment bypasses the canonical neosubstrate binding site on CRBN". This seems like an exaggeration. From the structure shown, it appears that G3BP2 in fact is primarily engaged by the IMiD binding pocket in the CULT domain. Authors argue that some residues lining the IMiD binding pocket in the CULT domain are not involved in binding to G3BP2 and G3BP2 in fact interacts with the LON domain of CRBN specifically with residues F150 and I152.

Response: MRT-5702 indeed binds to the same pocket as all known CRBN MGDs. The difference between G3BP2 and canonical neosubstrates/VAV1 is at the protein-protein interface where neosubstrates engage the CRBN neosurface. G-loops and VAV1 engage CRBN by forming hydrogen bond interactions with residues N351, H357 and W400 in the CRBN CULT domain. In fact, all MGD-induced ternary complex structures available in the PDB to date show these three residues in contact with the neosubstrate. The surprising finding in the G3BP2 ternary complex structure is that none of these three CRBN residues are contacted by G3BP2. Even more so, G3BP2 hardly forms any interactions with the CRBN CULT domain, but rather explores

extensive PPIs with the neighboring LON domain. To work out these differences more clearly, we decided to quantify the buried surface area (b.s.a.) between neosubstrates, the different CRBN domains (LON and CULT), and the MGD. These numbers show that while CK1a, ZNF692 and VAV1 form 0%, 2% and 7% of their contacts with the CRBN LON domain, G3BP2 forms 69% with the LON domain, a further 18% with the MGD and only 13% with the CULT domain (**Figure 5 and Extended Data Figure 10**). Moreover, G3BP2's interactions with the CULT domain are non-conventional and occur via the backside of the CULT's sensor loop, a different site than the one engaged by G-loops (**Figure 4c and Extended Data Figure 1d-f**). In summary, the Reviewer is correct in that the legend should read: "G3BP2 recruitment bypasses canonical protein-protein interactions between CRBN and G-loop-like motifs", which we changed in the current version.

To clarify this very important point we introduced the following changes:

- The introduction, results and discussion have been significantly adjusted to highlight the differences in the binding modes of G-loops, VAV1, and G3BP2.
- **Extended Data Fig. 1** has been added to highlight neosubstrate recruitment to CRBN including the critical interactions between G-loops and the key residues N351, H357 and W400
- **Figure 4** now shows three different views of the CRBN:MGD:G3BP2 interaction, and **EDF.9 c-f** provides the cryo-EM envelope of the respective regions that supports our interpretations (also see responses below)
- In the new **Figure 5**, we now provide a clear depiction of the interactions between CRBN, the G-loop target ZNF692, the G-loop surface mimic VAV1, and the non-G-loop target G3BP2, which highlights crucial differences at the PPI through multiple different representations.
- New **EDF. 10a** now shows footprints of multiple publicly available neosubstrates and their differentiation to the G3BP2 binding mode. **Figure 5d** and **EDF. 10b** now shows the total buried solvent excluded surface areas and the percentages for LON/CULT/MGD.

We hope that all these actions sufficiently address the Reviewer's previous concerns.

> *There are some concerns with these conclusions made by the authors based on the cryo-EM structure. See below*
> *2) The overall resolution of the cryo-EM structure is 2.5A. From the extended data figure 3 where the local resolution is projected onto the cryo-EM map, it is evident that most of the G3BP2 and the interface with CRBN is resolved to a much lower resolution of 3.5 to 4.5A (authors do mention this) where interpreting specific interactions becomes very tricky.*

Response: The Reviewer is correct in pointing out that the local resolution of the cryo-EM envelope of the full complex limits interpretation of specific side chain interactions at the ternary complex interface. As we recognized noticeable conformational flexibility at

the ternary complex interface of that structure, we decided to calculate a locally refined map of CRBN and the NTF2L homodimer which increased the overall resolution of this local reconstruction to 2.8 Å and provides detailed and interpretable insights into the ternary complex interface. For the locally refined map, we used particle subtraction followed by local refinement, which is a standard and well-validated protocol in the cryo-EM field to refine a selected sub-volume within a larger volume to high resolution (Bai et al, 2015; Ilca et al, 2015; Nguyen et al, 2016). Due to this heterogeneity, structure determination of CRBN/DDB1 ternary complexes usually requires the above mentioned approach to reveal the molecular details of the regions of interest, namely the glue-mediated interface of CRBN with the target proteins (Baek et al, 2024; Mercer et al, 2024; Hao et al, 2024; Shaum et al, 2024; Kwiatkowski et al 2025). We used this locally refined map for the interpretation of the ternary complex interface in the previous version of the manuscript (former EDF. 3) but fell short clarifying this in the previous version of the manuscript.

To address the Reviewer's points, we have introduced the following changes to the manuscript:

- We clarified usage of different maps for data interpretation in the main text (page 7, third paragraph).
- New **EDF. 4** (old **EDF. 3**) has been changed to better highlight the details of the cryo EM workflow and improved the visualization of the locally refined 2.8 Å map of the CRBN:G3BP2 interface.
- New **EDF. 5** now shows improved views on the observed density along key parts of the interface.
- New **EDF. 8** now shows model:density matches of the MRT-5702D ligand in two orientations.
- New **EDF. 9** now shows model:density matches of the CRBN:G3BP2 ternary complex interface.

> *For eg. the the Extended figure 4i shows the density of the ligand which seems to be of low resolution.*

Response: The resolution in the ligand area after computing the locally refined map is 2.8 Å.We improved the illustrations to better emphasize the density fit around the ligand:

- **EDF. 5h and 5i** now show two views of the density attributed to the compound.
- **EDF. 8e** now shows two views of the ligand:density fit.

> *Can the authors confirm if they see the full ligand bound because in this Extended figure 4i, only a part of it seems to be visible.*

Response: This is confirmed in the new panel in **Extended Data Fig. 8e**.

> *Figure 2F which is very important to support the main conclusion of the paper for eg. should include electron density to convince readers of the accuracy of fitting these residues at low resolution.*

- **Response:** New **EDF. 9** now shows model:density matches of the CRBN:MGD:G3BP2 ternary complex interface in four different views.

3) Do the residues F150 and I152 from the LON-loop also engage the glue?

Can the authors provide data to show their involvement (or the lack of it) in the binding of the glue? Did the authors consider the possibility that the loss of binding to G3BP2 by CRBN F150 and I152v mutants is perhaps due to the weakened glue binding to CRBN in general. Then the role of LON domain is only passive in the interaction between CRBN and G3BP2 as opposed to active role that is currently claimed.

Response: The cryoEM structure does not show any interaction between F150/I152 and the molecular glue, both of which are too far to interact (**Fig. 3a, Fig. 4b and Fig. 5c**). To further support this notion, we performed a NanoBRET TE experiment, an assay developed by PROMEGA to measure ligand binding to CRBN. We used this assay to test displacement of a fluorescent reporter compound (Tracer) from either CRBN WT or CRBN F150A by Lenalidomide or MRT-5702 and observed no effect on ligand binding (**Ext. Data. Fig. 3h, 3i**). Tracer binding to either CRBN versions, WT or F150, is identical (**Ext. Data Fig. 3g**), whereas a Trp-cage mutation, W386A, weakened binding by multiple orders of magnitude. Therefore, F150A does not impact compound binding but clearly impacts ternary complex formation. The manuscript was updated accordingly.

4) Authors also present a comparison of CRBN-G3BP2 structure with other structures in Figure 4. One of the structures shown is the complex of CRBN in complex with VAV1. Can the authors please clarify if this is a model generated or a cryo-EM structure? If it is already published, it would help the manuscript if the authors can discuss the novel aspects of CRBN-G3BP2 structure in comparison to CRBN-VAV1 complex structure which also lacks G-loop and which also engages the LON-loop in CRBN.

Response: Indeed, new **Fig. 5** (previously Fig. 4) compares to the structure of VAV1, which has now been published (PDB id: 9nfr) and is referenced accordingly in the revised version of the manuscript. VAV1 does not carry the structural recognition motif of the G-loop degron (β-hairpin or helical alpha-turn with a glycine residue in a key position), but was shown to mimic G-loop topology and electrostatics on a surface level. Because VAV1 represents a G-loop surface mimic, VAV1 can still engage the canonical G-loop binding site on CRBN through surface complementarity. We significantly improved the comparison between G3BP2, G-loop targets, and the G-loop surface mimic VAV1. Specifically, we introduced the following changes to address this Reviewer's comment:

- The revised introduction now contains an extended description of G-loop/VAV1 binding to residues N351, H357 and W500 of the CULT domain and this is further

illustrated by showing engagement of these three residues with previously identified β-hairpin G-loops, helical G-loops and the G-loop surface mimic VAV1 in the newly created **Extended Data Fig. 1**.

- Three new panels have been added to new **Figure 5** (previously Figure 4) depicting differences in the binding modes of G-loops, VAV1 and G3BP2. Furthermore, target footprints on CRBN have been improved to better visualize the crucial differences.
- **Extended Data Fig. 10** shows the footprints of multiple known CRBN molecular glue-dependent neosubstrates and are compared to the differentiated binding mode of G3BP2. A table has been added to **Extended Data Fig. 10** which quantifies the percentages of buried molecular surface deconvoluted for the different CRBN domains in the ternary complexes.

> *5) One of the last sentences in the manuscript reads "The rationalization of the novel G3BP2 binding mode suggests that MGD-induced protein contacts between CRBN and its neosubstrates rely on at least three determinants: (i) a preexisting PPI hotspot on either side of the interface, (ii) partial molecular mimicry of an endogenous PPI and (iii) complementation of these contacts by MGD-induced neointeractions." Authors present one structure of CRBN in complex with G3BP2, this alone is not enough to derive general conclusions about the CRBN binding to neosubstrates. Moreover the 3 points mentioned here are general principles that can be derived from many of the previous CRBN-glue-neosubstrate complex structures including the ground-breaking first structures with IMids.*

Response: We have addressed these points in our expanded and revised discussion. As the Reviewer points out, mimicry of endogenous interactions seems to be an emerging theme in molecular glues in general, an aspect that was long overlooked particularly for CRBN-based MGDs. Only recently it was clarified that G-loop-containing neosubstrates, and in particular helical G-loops, appear to mimic recruitment of an endogenous CRBN substrate (Ichikawa et al, 2002; Heim et al, 2022; Petzold et al, 2025). Remarkably, VAV1 mimics a G-loop-containing neosubstrate at the molecular surface level, maintaining three key hydrogen bonds with the CRBN CULT domain (residues N351, H357 and W400) (Petzold et al, 2025). Other cases of mimicry of endogenous PPIs related to glues have previously been reviewed (Schreiber et al, 2024).

Although our current study is limited to only one example, it shows the predictive power of the mimicry concept by rationalizing a novel neosubstrate binding mode on CRBN through the usage of preexisting PPI knowledge of a naturally occurring interaction (G3BP binding to an FGDF-motif). This represents a remarkable first case in which mimicry does not simply happen on the substrate level (endogenous substrate to neosubstrate), but instead the recognition logic is flipped and this time mimicry could be identified between an E3 and a natural binding partner of the G3BP2 neosubstrate,

Overall, I am fairly enthusiastic about the manuscript but the authors need to tone down some of the claims about the novelty and implications of the structure.

Response: We thank the Reviewer for these comments. The novelty has now been placed into context, contrasting to what was known about CRBN, and the generalization claims have been accordingly toned down.

Authors should also present their structure figures especially where the ligand and the surrounding areas are shown with the electron density clearly visible and in two different views.

Response: EDF. 5 (former EDF. 4), **EDF. 8** and **EDF. 9** now show significantly improved illustrations to address these comments. **EDF. 5h** and **EDF. 5i** present two views at 90^{o} angle of the ligand density. Moreover, **EDF. 8** now shows the cryo-EM envelope with the docked ligand in two orientations, and **EDF. 9c-f** show the ternary complex interface with the cryo-EM density to support our interpretations.

Reviewers #2*l*#3 (Remarks to the Author):

The manuscript by Annunziato et al. describes the characterisation of a novel recognition interface between CRBN and G3BP2 in the presence of a molecular glue degrader. Following an initial observation of potent and selective degradation of G3BP2 in a global proteomics experiment, the authors obtained genetic and biochemical evidence demonstrating that compound MRT-5702 induced formation of a ternary complex with CRBN and the neosubstrate. Protein domain mapping was used to determine that the NTF2L domain of G3BP2 was necessary and sufficient for this interaction to occur.
Since initial computational analyses of G3BP2 based on known CRBN degrons were unable to rationalise the binding, the authors hypothesised that CRBN could instead be mimicking a recognition motif for G3BP2. Structural similarities between the LON domain of CRBN and USP10 (a known interactor of G3BP2) were identified in computational modelling, suggesting the formation of a novel PPI at a pre-existing hotspot in a G-loop independent manner. This was supported experimentally with NanoBRET constructs, which indicated that mutations in the alternative binding regions reduced or abolished ternary complex formation. A cryo-EM structure of the complete ternary complex was obtained, and validated the predicted interface between CRBN, G3BP2 and MRT-5702. The four diastereomers of the MGD were isolated and tested separately via TR-FRET of ternary complex formation, demonstrating clear stereoselectivity of the interaction for a particular isomer, which was assigned based on crystallographic and computational data.

Overall, the study presents a clear and concise investigation into an interesting and novel interaction. It is clearly written and well presented, and the hypotheses and computational predictions are sufficiently supported by experimental

evidence. Given the broad interest in targeted protein degradation as a therapeutic modality, this study will be of great interest as it suggests a new protein interface on CRBN that can be accessed by molecular glues to mediate recognition of neosubstrates.
There are a number of points the authors should address to make the manuscript more easily accessible to the reader, which are listed below.

Response: We thank the Reviewers for the careful assessment of our manuscript and appreciate their evaluation of the work as clear and concise, novel, well presented, and of great interest. We are also grateful for the suggestions to further increase accessibility of the work to a broader readership, which we implemented in the revised version of this manuscript and are detailed below.

Major points
• Much more information is required in the introduction to provide proper context for the work. The broader implications of the study are currently unclear. What is already known about the CULT and LON domains of CRBN, including outside of the context of molecular glue degraders? Have endogenous binders or functions of the LON domain been identified? Detailed background information on CRBN, including an introductory figure on the structure and function of its domains, would put the study more clearly into context for readers (could be added to Extended Data).

Response: We thank the Reviewers for raising these very important points. In the revised version of the manuscript, we expanded the introduction to discuss prior knowledge on CRBN architecture and its endogenous functions. The role of the LON domain remains poorly understood, yet its homology to LON proteases suggests a contribution in substrate recognition, which has been added to the manuscript with appropriate references. An explanation of open and closed CRBN has been included, along with a new **Extended Data Fig. 1** that illustrates CRBN's architecture, its conformational changes upon MGD binding and the formation of an extended predicted PPI hotspot in closed CRBN.

• The introduction should also make a clear distinction where the previous work ends (e.g. the proteome-wide map of human CRBN target space, described in line 60) and the new results begin (presumably the global proteomics experiment in which G3BP2 degradation was first observed).

Response: The new introduction now clearly makes this distinction. Specifically, paragraph 4 of the introduction summarizes the previous work (Petzold et al, Science 2025) and contrasts it with the findings of this manuscript and its novelty.

• Please also introduce MRT-5702, how it was discovered, and some context for the global proteomics experiment in which G3BP2 degradation was observed.

Response: We updated the manuscript in the introduction section and results: "*We previously established a map of the CRBN target space through computational mining of G-loop-like motifs in the structural human proteome{Petzold, 2024 #86}. This map not only facilitates identification of G-loop-containing neosubstrates{Petzold, 2024 #86}, but also guides the prospective discovery of G-loop-independent targets in unbiased proteomic screens. Profiling a diversity selection of CRBN-centric MGDs in mass spectrometry (MS)-based global proteomics led to the identification of MRT-5702 (**Fig. 1a**), a dihydrouracil-containing compound that significantly reduced levels of Ras–GAP SH3 domain binding protein 2 (G3BP2; **Fig. 1b**), and to a lesser extent ubiquitin-specific peptidase 10 (USP10){Takayama, 2018 #116} in CAL51 cells after 6h compound treatment.*"

> *• Line 66: The authors report that MRT-5702 "potently reduces" levels of G3BP2 as measured by global proteomics. The study is missing a quantitative evaluation of potency of the molecular interactions and degradation; these would be of significant interest in the context of this novel CRBN protein-protein interaction. For example, DC50/Dmax for the compound should be estimated experimentally (e.g. based on NanoBRET, HiBiT, western blot, or global proteomics), and biophysical techniques (e.g. SPR) could be used to measure the cooperativity (α) of ternary complex formation.*

Response: We have included DC50/Dmax values for HiBiT-G3BP2 (**Fig. 1f**), TR-FRET EC50 (**Fig. 1i**) and NanoBRET EC50 (**Fig. 1d**). We also included these values for the active stereoisomer (MRT-5702D) in **Figure 2**. We also changed 'potently' to 'significantly' in the main text.

> *Minor points*
> *• In the abstract, the findings are described as a "generalisable concept". However, this manuscript describes an investigation into a single instance of a novel protein-protein interaction, so whether this is generalisable remains to be seen. Please adjust the statement.*

Response: We have removed this from the abstract and softened our conclusions with respect to generalization. We now mention the possibility that the emergent mimicry of naturally occurring interactions induced by molecular glues could evolve into a more generalizable underlying principle, and we also refer to other examples in the literature where molecular glue-induced interactions mimic endogenous interactions to support this statement.

> *• Figures containing protein structures could be more clearly presented:*
> *o The contrast in the colour schemes of protein chains in structural figures should be increased in order to clearly distinguish between different domains and proteins.*

Response: Contrast has been improved throughout all figures. The ligand is now colored in pink to contrast the G3BP2 and CRBN colors.

o In Extended Data Fig. 2a-2c, helices α1 & α2 have been misrendered as bent cylinders. This should be corrected for clarity and consistency with other figures.

Response: We thank the Reviewers for noticing this. Figures have been updated and corrected.

o The use of spheres to show specific amino acids or structural features is not very helpful. Ball and stick representation might be clearer to illustrate the importance of specific residues/contacts/elements.

Response: We have changed spheres to sticks in **Figures 3 and 4**, **EDF. 3**, **EDF. 8** and **EDF. 9**. All other figures use sticks with the exception of **Figure 5a-c** where we still use a spherical representation to provide a sense of 'packing' in the site, especially involving sensor loop interactions.

• Line 118: "deployed" doesn't make sense, should this be "recognised", "exploited", "bound"?

Response: We have changed 'deployed' to 'engaged'.

• Line 135: "Ablated" is an overstatement here - F150A mutation reduces binding of full-length wtG3BP2 but it is not completely lost, "reduced" would be more appropriate.

Response: We have changed 'ablated' to 'reduced'.

• In the supplementary methods, there is an error in the procedure for the final step in the synthesis of MRT-5702: the mass of the amine coupled to intermediate 8 does not match the moles/equivalents reported. The other steps of the synthesis should be proofread to check for similar errors.

Response: We thank the Reviewers for spotting this inconsistency. The entire section has been proof-read and errors have been corrected.

Additional points from the editor:

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

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-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures. Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. While these data can be displayed in a relatively informal style, they must refer back to the relevant figures. These data should be submitted as source data with the last revision, prior to acceptance.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.
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EXTENDED DATA FIGURES

When re-submitting your manuscript, please ensure that any supplementary figures and tables that are crucial to the manuscript's conclusions are converted into Extended Data

figures and tables to increase visibility of these data. Extended Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

Data availability: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. Deposition of deep

sequencing and microarray data is mandatory, and the datasets must be released prior to or upon publication. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

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