EMBO Molecular Medicine

The CB1 receptor interacts with cereblon and drives cereblon deficiency-associated memory shortfalls

Carlos Costas-Insua, Alba Hermoso-López, Estefanía Moreno, Carlos Montero-Fernández, Alicia Álvaro-Blázquez, Irene Maroto, Andrea Sánchez-Ruiz, Rebeca Diez-Alarcia, Cristina Blázquez, Paula Morales, Enric Canela, Vicent Casadó, Leyre Urigüen, Gertrudis Perea, Luigi Bellocchio, José Rodríguez-Crespo, and Manuel Guzmán

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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.)

24th Aug 2023

Dear Prof. Guzmán,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the three reviewers who agreed to evaluate your manuscript. As you will see from the reports below, the referees acknowledge the interest of the study and are overall supporting publication of your work pending appropriate revisions.

Addressing the reviewers' concerns in full will be necessary for further considering the manuscript in our journal, and acceptance of the manuscript will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

We are expecting your revised manuscript within three months, if you anticipate any delay, please contact us.

When submitting your revised manuscript, please carefully review the instructions that follow below. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

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.

2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF' (https://www.embopress.org/page/journal/17574684/authorguide#figureformat).

3) At EMBO Press we ask authors to provide source data for the main figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

4) 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.

5) A complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/17574684/authorguide#submissionofrevisions). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

7) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/17574684/authorguide#dataavailability).

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.
This study includes no data deposited in external repositories.

8) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.). Please provide exact p values.

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) 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.
- 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.
See detailed instructions here:

11) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting
- the medical issue you are addressing,
- the results obtained and
- their clinical impact.
This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

12) For more information: There is space at the end of each article to list relevant web links for further consultation by our readers. Could you identify some relevant ones and provide such information as well? Some examples are patient associations, relevant databases, OMIM/proteins/genes links, author's websites, etc...

13) Author contributions: CRediT has replaced the traditional author contributions section because it offers a systematic machine readable author contributions format that allows for more effective research assessment. Please remove the Authors Contributions from the manuscript and use the free text boxes beneath each contributing author's name in our system to add specific details on the author's contribution. More information is available in our guide to authors.

14) Disclosure statement and competing interests: We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary.

15) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high. Share synopsis text and image, as well as eTOC:
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16) As part of the EMBO Publications transparent editorial process initiative (see our Editorial at http://embomolmed.embopress.org/content/2/9/329), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts.
In the event of acceptance, this file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know whether you agree with the publication of the RPF and as here, if you want to remove or not any figures from it prior to publication.
Please note that the Authors checklist will be published at the end of the RPF.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

I look forward to receiving your revised manuscript.

Yours sincerely,

Poonam Bheda

Poonam Bheda, PhD
Scientific Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

This is an excellent report and my comment are outlined below.

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Concerns to address
•Line 228: here specify how you measure cAMP and if IBMX is added to measure cAMP accumulation.
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Suggested experiments, minor comments, and edits.
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•Line 251: delete "Aside from these cell-signalling experiments" and "in further detail"

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In this paper, the authors examined the role of cereblon (CRBN) in neural function, using cell type-specific KO mice. They found at the molecular level that CRBN expressed in excitatory neurons was required for maintenance of normal neural function by inhibiting the cannabinoid receptor CB1 (CB1R) pathway. At the whole-animal level, mutant mice lacking CRBN in excitatory neurons showed memory deficit, which was recovered by the CB1R-specific antagonist. Although the elaborated analyses on the role for CRBN in neural functions at the molecular level and the whole-animal level should be highly appreciated, there is a large black box between the two levels.

Major comments:
(1) The authors should at least try to elucidate the mechanism at the synaptic level. For instance, they can analyze the phenotypes of the mutant mice using acute brain slices or cultured neurons. If the authors can show that the CB1R pathway is enhanced in mutant mice electrophysiologically, it would strengthen the conclusions of this paper considerably. Such data would attract many readers of EMBO Molecular Medicine.
(2) Fig. 2D: The result of the motor coordination (the first trial of the rotarod test) should also be shown (or described in the text). The result of motor learning would be meaningless if the motor coordination is impaired.
(3) Fig. 2F, 2I: The authors should analyze the data by using "two"-way ANOVA, because there are two groups (WT and KO) with two different conditions (O and M for Fig. 2F; N and F for Fig. 2I). Since there seem to be some other problems in statistical analyses throughout the paper, all the data should be reexamined by a person who specializes in statistics.
(4) Fig. 1B, 1C: Why were the 4 groups (CRBN-WT, Glu-CRBN-KO, GABA-CRBN-KO and CRBN-KO) compared here? In the other experiments, the data of WT and KO mice were compared in each genotype (CRBN-KO, Glu-CRBN-KO and GABA-CRBN-KO).

Minor comments:
(5) Throughout the study, is there any statistical difference between the data of male and female mice?

Referee #3 (Comments on Novelty/Model System for Author):

The authors have performed laborious in vitro and in vivo experiments, uncovering the unexplored role of CRBN for CB1R-mediated regulation of brain function. The conceptual novelty of these findings is significant and would be of interest for a broad audience in the field of developmental brain disorders. The manuscript is well-written; however, several weaknesses need be addressed to enhance the rigor and overall quality of the study.

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In this manuscript, Costas-Insua et al. report on the role of CRBN, a genetic risk factor for intellectual disability, in maintaining memory function. Using multiple CRBN deletion mouse models, the authors have identified that CRBN, particularly expressed in glutamatergic neurons, regulates memory function. Mechanistically, the authors reveal that CRBN interacts with CB1R, impeding the CB1R-Gi/o-cAMP-PKA pathway in a manner independent of its ubiquitin ligase function. These mechanisms may underlie the cognitive impact of CRBN, as memory deficits resulting from genetic CRBN deletion were alleviated by the acute pharmacological blockade of CB1R.
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A major weakness in the present study is the absence of physiological data that reveal that genetic deletion of CRBN accelerates CB1R-mediated suppression of glutamatergic neuronal function, which could be rescued by the CB1R inhibition. Without this data, it remains unclear whether CRBN-CB1R interaction is a critical regulator for glutamatergic neurons. The author should provide this evidence by performing electrophysiological assays, such as brain slice recording experiments.

Other weaknesses include:

The authors used the Y-maze test to assess special memory. However, the Y-maze test predominantly measures habituation processes (rather than memory, as widely assumed. For a detailed discussion see: Sanderson et al. Neuropsychologia 2010;48(8):2303-15; Sanderson and Bannerman, Hippocampus 2012;22(5):981-94).

The forced swim test was used to evaluate depression. Nevertheless, while the forced swim test is still used to characterizing the efficacy of antidepressants, the field recognizes that it is unclear whether the observed phenotypes are valid correlates of depressive symptoms. The authors should rephrase the result section accordingly.

To evaluate CB1R functionality in CRBN-deficient mice, the authors examined the impact of THC treatment on catalepsy. However, cognitive effects of THC have been extensively studied in the literature. Studying cognitive tasks is more appropriate for the current study.

Despite the inclusion of both male and female mice in the study, it is uncertian if any sex differences exist. While sex difference is not the primary scope of the current study, the authors should at least analyze some data separately for male and female mice and discuss potential sex effects. It is worth noting that certain studies have reported sex differences in the effects of THC. Perhaps clinical studies may also reveal sex differences in CRBN mutations genetically associated with intellectual disability?

Minor point:
Page 11, line 267. Please spell out what "PLA" is.

POINT BY POINT RESPONSE TO THE REFEREES' REPORTS

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We would like to thank the reviewer for his/her positive and constructive comments, which have helped to improve the quality of our study.

Concerns to address

•Line 228: here specify how you measure cAMP and if IBMX is added to measure cAMP accumulation.

Done.

•Line 233: "PKA inactivation, an effect that was also prevented by CRBN" this statement is unclear. Are the authors referring to "reduced PKA activation"? Please clarify, and here specify the assay that was employed to measure PKA and time point of measure.

Done (see also Materials and Methods, "Determination of PKA activity").

•Line 234: Are the authors refereeing to "a response that was absent when expressing CRBN"? Please clarify.

Done.

•Line 238: Specify the assay.

Done.

•Line 245: "CB1R function in HEK-293T cells". Were these cells transiently transfected or stable cell line? Please clarify.

Done.

•Line 247: I do not understand this conclusion and do not see any statistical differences in the results. Please explain / elaborate.

Done.

•Line 293: Replace "blockade" by "antagonism"

Done.

•Line 348: After "CRBN", add "that we report here on CB1R-cAMP signaling"

Done.

•Line 353: Provide background and relevance for discussing AHA1.

Done.

•Line 803 Determination of PKA activity. Is this a copy past error as it's already described earlier.

Done (removed from "Cell culture, transfection and signalling experiments"; sorry for the confusion).

•Figure 2A: Were all mice weighted exactly at P60, especially considering line 620: "Adult mice (2-4-month-old)"? If not, please add the range. Also, specify in the manuscript if both or only one sex was studied.

Corrected in Fig 2AB and the corresponding figure legends. Animals of both sexes were used and analyzed as disaggregated for sex (see Materials and Methods, "Animals", lines 436-438; and Appendix Table S1).

•Figures 5E and F: "60 s" out of how long total?

The scale of the y-axis was cut at 60 s because this was the maximal duration of the catalepsy test. This has been clarified in the legend to Figs 5EF (now FG; see also Materials and Methods, "Cannabinoid administration", line 666).

Suggested experiments, minor comments, and edits.
•Figure 5B: The results show that only a small amount of CB1R is associated with CRBN. Could it be that the lower expression CB1R in Glut subpopulation are binding to CRBN and not the more abundant CB1R in GABA? It would be interesting to extend this result with a similar experiment on the CRBN mouse line and include this new result in the study.

We agree with the reviewer. This is indeed a very likely possibility. To test it, we used the PLA technique to allow a sensitive CB_1R-CRBN direct-interaction/close-proximity assessment in brain slices *in situ*. These new PLA experiments showed abundant fluorescence-positive *puncta* in WT and GABA-CB_1R-KO mice, but not in Glu-CB_1R-KO and CB_1R-KO animals (Fig 5C; text, lines 278-281), hence further supporting the CB_1R-CRBN association in hippocampal glutamatergic but not GABAergic neurons.

•Line 46: replace "the blockade of CB1R" by "CB1R antagonists".
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We have incorporated all the changes suggested by the reviewer.

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We agree with the reviewer. We have therefore conducted electrophysiology experiments in hippocampal slices and measured synaptic plasticity-associated parameters. As previous studies had already investigated the electrophysiological alterations occurring in hippocampal slices from CRBN-KO mice (*cf.* Bavley *et al.*, 2018, Choi *et al.*, 2018; both cited in the paper), we sought to analyze -as for G protein coupling (Fig 5D)- the effect of viral vector-driven CRBN overexpression in CA1 hippocampal neurons. We measured two archetypical forms of endocannabinoid-mediated short-term synaptic plasticity, namely a depolarization-induced suppression of excitation (DSE) and inhibition (DSI), which rely on the CB_1R-dependent control of glutamatergic and GABAergic transmission, respectively. As shown in new Fig 5E, CRBN overexpression blunted DSE but had no effect on DSI. Taken together, these electrophysiology data provide further support to the notion that CRBN *in vivo* blunts CB_1R activation selectively at glutamatergic terminals. See also Results (lines 286-300) and Materials and Methods (*Ex vivo* electrophysiological recordings, lines 852-888).

(2) Fig. 2D: The result of the motor coordination (the first trial of the rotarod test) should also be shown (or described in the text). The result of motor learning would be meaningless if the motor coordination is impaired.

We agree with the reviewer. Now we also show the data of the three days tested by separate in Fig EV2A (see Results, line 152; and explanation of the rotarod test in Materials and Methods, "Motor performance tests", lines 452-459).

(3) Fig. 2F, 2I: The authors should analyze the data by using "two"-way ANOVA, because there are two groups (WT and KO) with two different conditions (O and M for Fig. 2F; N and F for Fig. 2I). Since there seem to be some other problems in statistical analyses throughout the paper, all the data should be reexamined by a person who specializes in statistics.

We agree with the reviewer. Sorry for this error. Those data have now been reanalyzed by two-way ANOVA. We have reviewed all the statistical analyses in the paper.

(4) Fig. 1B, 1C: Why were the 4 groups (CRBN-WT, Glu-CRBN-KO, GABA-CRBN-KO and CRBN-KO) compared here? In the other experiments, the data of WT and KO mice were compared in each genotype (CRBN-KO, Glu-CRBN-KO and GABA-CRBN-KO).

We agree with this remark. All the behavioral tests were conducted with KO (Cre+) and WT/floxed (Cre-) littermates within each mouse line because, owing to the high inter-individual variability of these tests, they demand high *n* values for an appropriate statistical analysis to be conducted. From a logistical standpoint, these tests can be readily performed with many animals in our laboratory. Regarding the RNAscope technique, on the one hand, the analysis is very laborious within each animal. On the other hand, we obtained essentially all-or-none, low variability *Crbn*-transcript expression data in the different brain regions of the three KO lines. Hence, we decided to pool the 6 WT/floxed mice used (2 littermates from the Glu-CRBN-KO colony, 2 littermates from the GABA-CRBN-KO colony, and 2 littermates from the CRBN-KO colony) into a single WT/floxed group. Please note that all these WT/floxed mice share an identical genotype (*Crbn*$^{fl/fl,Cre-}$).

Minor comments:
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Both male and female mice were used in the study, and specific symbols for each of the two sexes are shown where appropriate. Source data were collected and analyzed as disaggregated for sex, but that information was not included in the previous version of the manuscript. Please find it now in the Appendix. We increased the n values of some of the experimental groups to make the statistical analyses more robust. Except -as expected- for body weight, that was slightly higher in males than in females (Fig 2A), no gross sex-specific differences were found in the numerous parameters measured. We are nonetheless aware that statistical trends appeared in a few cases, and we cannot rule out that sample size was not high enough to enable meaningful *post hoc* statistical conclusions.

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We agree with the reviewer that the standard Y-maze test predominantly assesses short-term habituation to recently experienced stimuli. Nonetheless, here we used a modified version of the test that has been reported to largely measure hippocampal-dependent spatial reference memory (Kraeuter *et al.*, 2019). We have rephrased in the text the outcome evaluated by this test (lines 167-168).

The forced swim test was used to evaluate depression. Nevertheless, while the forced swim test is still used to characterizing the efficacy of antidepressants, the field recognizes that it is unclear whether the observed phenotypes are valid correlates of depressive symptoms. The authors should rephrase the result section accordingly.

The reviewer is correct. We have rephrased in the text the outcome measured by this test (lines 160-161).

To evaluate CB1R functionality in CRBN-deficient mice, the authors examined the impact of THC treatment on catalepsy. However, cognitive effects of THC have been extensively studied in the literature. Studying cognitive tasks is more appropriate for the current study.

We understand the reviewer's comment. As he/she points out, the aim of these experiments was to assess CB_1R functionality in CRBN-deficient mice by measuring the behavioral response of the animals to a submaximal dose of a cannabinoid agonist (3 mg/kg THC). However, please note that if we had measured cognitive tasks, it would have been hardly feasible to evaluate CB_1R functionality as the cognitive shortfalls that are induced by THC administration are already present in CRBN-deficient animals. For example, in the NOR test, neither CRBN-KO nor Glu-CRBN-KO mice discriminated basally between the new and familiar objects (Figs 2H and 6A; discrimination indexes around 0). Hence, conceivably, this bad performance could not be worsened even further by agonizing the CB_1R with THC. In fact, the memory deficits shown by CRBN-KO and Glu-CRBN-KO mice were rescued by antagonizing the CB_1R with rimonabant (Fig 6A). We consequently sought to evaluate behavioral traits that *i*) are unaffected basally by CRBN deficiency, *ii*) are evoked in a straightforward manner by a CB_1R agonist, and *iii*) can be measured rapidly and unambiguously. So, we decided to assess catalepsy and thermal analgesia.

Despite the inclusion of both male and female mice in the study, it is uncertian if any sex differences exist. While sex difference is not the primary scope of the current study, the authors should at least analyze some data separately for male and female mice and discuss potential sex effects. It is worth noting that certain studies have reported sex differences in the effects of THC. Perhaps clinical studies may also reveal sex differences in CRBN mutations genetically associated with intellectual disability?

Both male and female mice were used in the study, and specific symbols for each of the two sexes are shown where appropriate. Source data were collected and analyzed as disaggregated for sex, but that information was not included in

the previous version of the manuscript. Please find it now in the Appendix. We increased the n values of some of the experimental groups to make the statistical analyses more robust. Except -as expected- for body weight, that was slightly higher in males than in females (Fig 2A), no gross sex-specific differences were found in the numerous parameters measured. We are nonetheless aware that statistical trends appeared in a few cases, and we cannot rule out that sample size was not high enough to enable meaningful *post hoc* statistical conclusions.

Minor point:
Page 11, line 267. Please spell out what "PLA" is.

We have spelled out the abbreviation when first used in the text (line 200).

22nd Feb 2024

Dear Prof. Guzmán,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Please place individual sections of the manuscript in the following order: Title page - Abstract & Keywords - Introduction - Results - Discussion - Materials & Methods - Data Availability - Acknowledgements - Disclosure and Competing Interests Statement - The Paper Explained - For More Information - References - Figure Legends - Expanded View Figure Legends.
- Please move the Acknowledgements to after the Data Availability statement.
2) Please ensure that the sequence for primers used for genotyping in Figure 1A are included in the Materials & Methods.
3) In the Appendix file, please include page numbers in the table of contents. We would also suggest that you rotate the tables so that they can be more easily read - this should be okay even if the text is small as readers should be able to zoom in if the table is of high enough resolution. However, we will leave that up to your discretion.
4) Please ensure that all funding sources are entered into the manuscript submission system (i.e. please add FPU16/02593 and FPU15/01833)
5) Please ensure that the synopsis image is uploaded as a high-resolution jpeg, TIFF, or png file 550 pixels wide x (250-400) pixels high. Currently the figure is provided as a PDF.
6) Please check your synopsis text and image before submission with your revised manuscript. Please be aware that in the proof stage minor corrections only are allowed (e.g., typos).
7) Please ensure that a completed Source Data checklist is uploaded - no checklist was included in your previous submission. You should have received the checklist from Dr. Hannah Sonntag to complete and include with your resubmission. Please also upload an individual source data file (zipped) per main figure - currently these are zipped together. The source data file for the EV figures can remain in a single zipped folder.
8) As part of the EMBO Publications transparent editorial process initiative (see our policy here: https://www.embopress.org/transparent-process#Review_Process), EMBO Molecular Medicine will publish online a Peer Review File (PRF) to accompany accepted manuscripts. This file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know whether you agree with the publication of the PRF and as here, if you want to remove or not any figures from it prior to publication. Please note that the Authors checklist will be published at the end of the PRF.
9) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file).

I look forward to reading a new revised version of your manuscript as soon as possible.

Sincerely,

Poonam

Poonam Bheda, PhD
Scientific Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

All my concerns have been addressed.

Referee #2 (Remarks for Author):

This paper has been improved considerably, and I have no more comments.

Referee #3 (Remarks for Author):

The authors adequately responded all concerns from this reviewer.

All editorial and formatting issues were resolved by the authors.

5th Mar 2024

Dear Prof. Guzmán,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

Your manuscript will be processed for publication by EMBO Press. It will be copy edited and you will receive page proofs prior to publication. Please note that you will be contacted by Springer Nature Author Services to complete licensing and payment information.

You may qualify for financial assistance for your publication charges - either via a Springer Nature fully open access agreement or an EMBO initiative. Check your eligibility: https://www.embopress.org/page/journal/17574684/authorguide#chargesguide

Should you be planning a Press Release on your article, please get in contact with embo_production@springernature.com as early as possible in order to coordinate publication and release dates.

If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to EMBO Molecular Medicine.

Yours sincerely,

Poonam Bheda, PhD
Scientific Editor
EMBO Molecular Medicine

>>> Please note that it is EMBO Molecular Medicine policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://www.embopress.org/transparent-process#Review_Process

EMBO Press Author Checklist

Corresponding Author Name: Manuel Guzmán	**USEFUL LINKS FOR COMPLETING THIS FORM**
Journal Submitted to: EMBO Molecular Medicine	The EMBO Journal - Author Guidelines
Manuscript Number: EMM-2023-18398	EMBO Reports - Author Guidelines
	Molecular Systems Biology - Author Guidelines
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Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	CRBN-KO, Glu-CRBN-KO and GABA-CRBN-KO mice (Materials and Methods: Animals; Results: Selective genetic inactivation of Crbn in glutamatergic neurons impairs memory). CRBN-expressing plasmids

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	The following primary and secondary antibodies (with their dilutions, applications, suppliers, and catalog numbers) were used in this study (Materials and Methods: RNAscope and immunofluorescence, Proximity ligation assay (PLA), Western blot and immunoprecipitation, Antibody-capture [35S]GTPγS scintillation proximity assay). All antibodies were used according

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	The sequences of the Crbn primers (Materials and Methods: RNA isolation and quantitative PCR) and the Crbn-targeted siRNA (Materials and Methods: CRBN knock-down) are provided.

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	HEK-293T cell line (American Type Culture Collection, #CRL-3216; Materials and Methods: Cell culture, transfection and signalling experiments)
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	The HEK-293T cell line was not recently authenticated and was negative for mycoplasma contamination (Materials and Methods: Cell culture, transfection and signalling experiments).

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Species: Mus musculus; strain: C57BL/6N; sex: male and female (at approximately 1:1 ratio); age: 8-14 wk; genetic modifications: Crbn-floxed/floxed;CMV-Cre (CRBN-KO), Crbn-floxed/floxed;Nex1-Cre (Glu-CRBN-KO), Crbn-floxed/floxed;Dlx5/6-Cre (GABA-CRBN-KO), Cnr1-floxed/floxed;CMV-Cre (CB1R-KO), Cnr1-floxed/floxed;Nex1-Cre (Glu-CB1R-
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Throughout the study, animals had unrestricted access to food and water. They were housed (typically, 4-5 mice per cage) under controlled temperature

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	We thank the personnel of the core microscopy centre, the genomics unit, and the animal facilities of Universidad Complutense de Madrid for their expert technical assistance (Acknowledgements).

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	**Yes**	The sample size for each experiment was estimated based on previous studies conducted by our laboratories using similar in vitro and in vivo models (Materials and Methods: Experimental design and statistical analyses).
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	**Yes**	In all experiments, biological samples (cultured cells, tissue extracts, brain sections) and animals (mice) were allocated randomly into the different groups (Materials and Methods: Experimental design and statistical analyses).
Include a statement about **blinding** even if no blinding was done.	**Yes**	In vivo experiments were routinely performed and analysed in a blinded manner for mouse genotype, viral injection, and pharmacological treatment (typically, an experimenter prepared the animals and the samples derived from
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	**Yes**	No data were excluded for the statistical analyses except when, very rarely, it was obvious that a technical problem had occurred in the measure (Materials and Methods: Experimental design and statistical analyses).
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	**Yes**	The statistical tests that were applied for each dataset are indicated in each figure legend. All datasets were tested for normality and homoscedasticity prior to analysis (Materials and Methods: Experimental design and statistical analyses).

Sample definition and in-laboratory replication	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	**Yes**	The number of biological replicates (number of mice, number of mouse hippocampal preparations, number of cellular experiments, number of subcellular experiments) is provided in each figure legend (Materials and Methods: Experimental design and statistical analyses).
In the figure legends: define whether data describe **technical or biological replicates**.	**Yes**	The number of biological replicates (number of mice, number of mouse hippocampal preparations, number of cellular experiments, number of subcellular experiments) is provided in each figure legend. The number of technical replicates is provided in the corresponding Materials and Methods subsection (Materials and Methods: Experimental design and statistical analyses).

Ethics

Ethics	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: 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	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	**Yes**	All the experimental procedures used were performed in accordance with the guidelines and with the approval of the Animal Welfare Committee of Universidad Complutense de Madrid and Comunidad de Madrid (protocol codes PROEX 209/18 and PROEX 032.0/22), and in accordance with the directives of the European Commission (Materials and Methods: Animals)
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	**Yes**	The ARRIVE guidelines were followed as closely as possible (Materials and Methods: Animals).
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	