EMBO reports

Absence of RNA binding protein FXR2P prevents prolonged phase of kainate-induced seizures

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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.)

1st Editorial Decision 7th Sep 2020

Dear Claudia,

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. We have now received the enclosed referee reports on it.

As you will see, all referees acknowledge that the findings are interesting.
However, they all also have suggestions for how the data and the study should be strengthened. I think that all concerns make sense and should be addressed, however, please let me know if you disagree, and we can certainly talk about the revisions in more detail in a video chat or telephone call, if you like.

I would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate.

Regarding data quantification, please specify the number "n" for how many independent experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

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

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).

See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

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

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- 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.

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/14693178/authorguide>. 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 (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please remember to provide a reviewer password if the datasets are not yet public. The accession numbers and database should be listed in a formal "Data Availability" section placed after Materials & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

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

<https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

9) Our journal also 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 https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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

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

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

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

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

This paper, "Absence of the RNA binding protein FXR2P protects against status epilepticus" by Lo et al. describes a potential role of the FXR2 (a paralogue of the intellectual disability/autism gene FMR1) protein product in epilepsy. Inspired by the observation that patients reported in public databases with a deletion of this gene never have epilepsy in contrast to comparable deletions in the region. Continuing on an FXR2 knockout mouse model, the authors show a much reduced status epilepticus after kainite but not pilocarpine induction. Interestingly, IP experiments show an overrepresentatation of glutamatergic components bound to FXR2P. Genes already shown to be involved in epilepsy are also statistically significantly overrepresented in this gene set. In order to substantiate their hypothesis that glutamatergic signaling is somehow compromised in the FXR2 knockout model, underexpression of two glutamatergic receptors is demonstrated and a decrease of phosphorylated ERK1/2 following kainate induction. The latter provides a plausible rationale for

the absence of the status epilepticus in the FXR2 mouse model.

This is a very well written paper that provides arguments that a loss of function of the FXR2 gene in humans protects against status epilepticus and proves this concept in an animal model along with a well augmented mechanistic hypothesis. Protective genes may be as frequent as disease causing genes, yet a protective role for a gene has been very rarely described, making this a relatively unique work. I do have some comments.

Major comments:

In the description of the deletion patients, only male patients are mentioned. Were there no females with deletions? This seems unlikely to me. Why were females not included? Please provide a rationale.

The description of the mouse model in the materials and methods section falls short. Three different genetics backgrounds are mentioned for the knockout, 129, B6 and FVB. Given the enormous difference in seizure susceptibility between these strains, please provide some more information on how the colony was maintained. I realize the colony was created with the 3 backgrounds, but as this was almost two decades ago, it makes a big difference how this one was maintained (by backcross with B6, FVB, other?). As for the wild type animals, where were these purchased and were these B6 or FVB.

Minor comments:

Introduction:
"...epilepsies, often referred to as epileptic encephalopathies."
This in not entirely true, epileptic encephalopathies refer to syndromic and severe forms of epilepsies, not to all.

"both individuals with Fragile X syndrome (FXS) and mouse models for FXS show increased seizure susceptibility"
Rather, individuals have an increased seizure frequency, whereas mice show increase susceptibility to audiogenic seizure induction.

"This difference can be attributed to elevated levels of ERK1/2 (extracellular kinase 1/2) phosphorylation and altered translation of specific mRNAs"
This has been postulated, but in my opinion not enough evidence has been provided in the papers referred to (that deal with human patient studies only) to substantiate this claim. Better mention as an hypothesis

Results:

1st par. I would suggest to add one/two sentences of explanation on the Open Targets Platform, as many readers may not be familiar with this platform and its potential.

2nd par on the FXR2 knockout.
The model is hardly introduced. What are the characteristics, arehomozygous or heterozygous knockouts used. What is known from this model? Is it known to display seizures? Has this been tested at all. In addition, The mice are generated from heterozygous intercrosses, were the full knockouts or the heterozygous knockouts used, or both.

3rd par on transcriptome
"Genes that were differentially expressed"
Rather, diffentially bound by the FXR2P

4th par on mRNA targets
Please mention GluK5 as a kainate receptor when first mentioning this in the text

Discussion
"We therefore propose that FXR2P is responsible for the mRNA translation....."
Though the authors unequivocally demonstrated the involvement of FXR2P in the glutamatergic signaling, I do not find arguments in this work to narrow this down to the level of RNA translation

Please include a few sentences on the outcome of the MEA recordings in the conclusion.

General:
Not sure that epilepsy can be named a "behavioral" outcome. Also I believe the name "behavioral epilepsy" is not widely accepted and I would suggest to avoid this term

Referee #2:

This study by Lo et al. investigated the roles of an RNA-binding protein FXR2P in neuronal excitability regulation and seizure susceptibility. The authors show that knocking out FXR2P leads to reduced seizure susceptibility presumably through ERK1/2 associated signaling. Strengths of this manuscript include good quality of the data, the use of proper controls and nice flow of the written document. However, while the data are properly presented and analyzed, the study is very preliminary. The main conclusion is built upon some superficial observation and is not supported by the data, which led to many overstatements. Many alternative mechanisms were not considered. Overall, the impact of this study is limited with its current data.

Major points:
1. The authors claimed multiple times throughout the manuscript that their data suggest the role of FXR2P in the development of status epilepticus (SE). This is not an appropriate statement. The data in this manuscript only indicate reduced excitability or seizure threshold in FXR2P knockout mice/neurons, which does not reflect SE in any way. Unless the author monitored chronic spontaneous seizures, it is inappropriate the suggest FXR2P affects SE.

2. The authors showed that ERK1/2 signaling is altered in FXR2P KO mice and suggest that this is the underlying mechanism following GluK5 activation and altered seizure activity. Many factors can cause activation of ERK1/2. Without experiments to evaluate GluK5, how can the authors conclude that the effect is through GluK5 as suggested in Fig. 5D? The effect could very likely be a secondary effect following KA-induced hyperactivity in the brain.

3. What is the mortality rate in the seizure experiments? Pilocarpine is especially known to elicit high mortality. The authors should provide information about the mortality in their experiments.

4. The authors' model suggests that FXR2P's function primarily occurs later during the seizure progression. Does the expression of FXR2P's targets (mGluR5, PSD-95 etc.) truly follow the same

time course as ERK1/2 activation after KA injection (Fig. 4D) and, if yes, does that depend on FXR2P? Does FXR2P expression change over the course of seizure progression?

5. The authors nicely showed reduced seizure activity in FXR2P KO mice. However, the MEA data in Fig. 5 does not provide much beyond that. It is unclear why Picrotoxin had to be added to increase neuronal firing. Can the author rule out any possibility that FXR2P KO and WT mice respond differently to Picrotoxin? Would it be possible that GABAergic signaling is altered in FXR2P KO mice that led to the observation in Fig. 5A? If the authors really need stimulation, why not use KA?

6. Related to point #4, it would be more informative if the authors can compare basal activity and after KA stimulation. This would tell us whether the effect is truly associated with KA or the neurons are basally more excited to begin with. It would also be better if a time course as in Fig. 4 can be applied. MEA is a power tool. It is a pity that the authors did not take advantages of this system to obtain more information.

7. Since FXR2P seems to regulate the expression of many regulators associated with glutamatergic signaling (Fig. 3), it would be useful if they authors can also evaluate synaptic transmission rather than only MEA recording.

Referee #3:

In the present manuscript, Bagni and colleagues investigate the role of the RBP FXR2P, a member of the family of Fragile-X-related proteins, in epilepsy. They report that the genetic loss of FXRP2 in mice reduces specifically the responsiveness to kainate, but not pilocarpine-induced epileptic seizures. By RIP-seq, they identify hundreds of mRNAs that are bound by FXR2P in the mouse brain, in particular those associated with glutamatergic synaptic transmission. Downregulation of these components in FXR2P ko mice correlates with the inability to sustain ERK activation after kainate. FXR2P ko slices show reduced burst activity compared to wt slices in response to picrotoxin. The authors propose a model whereby FXR2P stabilizes important components of glutamatergic synapses, thereby gating seizure activity after excessive stimulation of glutamatergic signalling.
Overall, this is a well conceptualized study that provides novel insight regarding the role of FXR2P in the regulation of epileptic seizures. Before these results can be published, the authors should revise their manuscript according to my comments below.

Specific comments:
1. The RIP-seq experiments needs more validation. An independent RNA-IP followed by qPCR for some candidates should be performed. In general, it was unclear why the p-value for the differential analysis between WT and KO was set to 0.1. According to the gene lists provided, an adjusted p-value of 0.05 would have already yielded >400 genes.
2. Some more detailed description of the GO-term analysis is required. For example, what was the background gene list used? Using results from the KO appears problematic, since the genes recovered from those IPs only represent non-specific binders. A better background would be the complete list of genes which are actually expressed in the system (e.g. determined by standard polyA RNA-seq).
3. From the few candidates that were followed-up, it appears that all of them are downregulated at

the protein level in the absence of FXR2P. Is this also true for the respective mRNAs? Does FXR2P in general stabilize target mRNAs, or is the regulation rather happening at the level of mRNA translation? Is there an enrichment of binding motifs for FXR2P in the RIP-targets, e.g. in the 3'UTR? Some additional mechanistic insight regarding the function of FXR2P in target gene regulation would be desirable.
4. The overlap between epilepsy genes and FXR2P targets appears substantial, but is this overlap really more than expected by chance? What statistical test was used to draw this conclusion?
5. Why were the number of slices different for WT and KO in the MEA recordings? It would also be important to report the number of individual animals these slices were derived from.
6. What is the effect of FXR2P knockout on basal glutamatergic transmission? This could be for addressed by patch-clamp electrophysiological recordings of mEPSCs in hippocampal slices.

Point by Point to "Absence of the RNA binding protein FXR2P protects against status epilepticus" by Lo et al. EMBOR-2020-51404V1

We would like to thank the referees' appreciation of our work and valuable suggestions. Based on their helpful comments, advice and requests we have revised the manuscript adding new data – compatibly with the COVID limitations.

We now provide a point by point and a revised version of the manuscript.

In brief we have:

1. Added *new panel in Figure 3 (3E)*
2. Added *new panel in Figure 4 (4C).*
3. Added *new panel in Figure 5 (5B)*
4. Revised *Figure 5D - now Figure 7*
5. Added *new Figure EV1*
6. Added *new panel in Figure EV2 (2B-C)*
7. Added new *Figure EV4*
8. Added *Figure 1 for reviewer #2*
9. Revised *Table EV1*

And modified the text accordingly.

Referee #1:

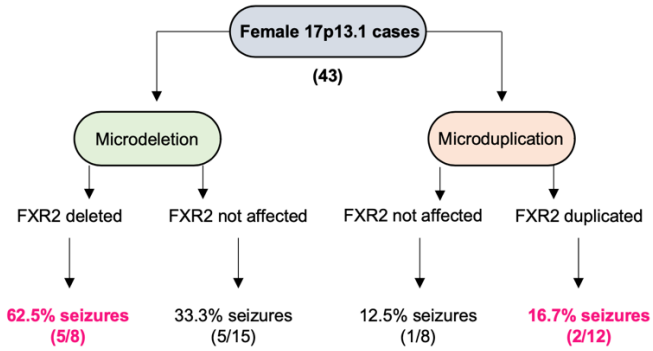
This paper, "Absence of the RNA binding protein FXR2P protects against status epilepticus" by Lo et al. describes a potential role of the FXR2 (a paralogue of the intellectual disability/autism gene FMR1) protein product in epilepsy. Inspired by the observation that patients reported in public databases with a deletion of this gene never have epilepsy in contrast to comparable deletions in the region. Continuing on an FXR2 knockout mouse model, the authors show a much reduced status epilepticus after kainite but not pilocarpine induction. Interestingly, IP experiments show an overrepresentatation of glutamatergic components bound to FXR2P. Genes already shown to be involved in epilepsy are also statistically significantly overrepresented in this gene set. In order to substantiate their hypothesis that glutamatergic signaling is somehow compromised in the FXR2 knockout model, underexpression of two glutamatergic receptors is demonstrated and a decrease of phosphorylated ERK1/2 following kainate induction. The latter provides a plausible rationale for the absence of the status epilepticus in the FXR2 mouse model.

This is a very well written paper that provides arguments that a loss of function of the FXR2 gene in humans protects against status epilepticus and proves this concept in an animal model along with a well augmented mechanistic hypothesis. Protective genes may be as frequent as disease causing genes, yet a protective role for a gene has been very rarely described, making this a relatively unique work. I do have some comments.

Major comments:

In the description of the deletion patients, only male patients are mentioned. Were there no females with deletions? This seems unlikely to me. Why were females not included? Please provide a rationale.
We appreciate this comment, and the reviewer is correct: we can find females with 17p13.1 deletions or duplications on the DECIPHER database. We have now added those data to the

revised manuscript (*new Figure EV1*). Of note, in this case, deletions were not associated with absence of seizures (see Figure below).

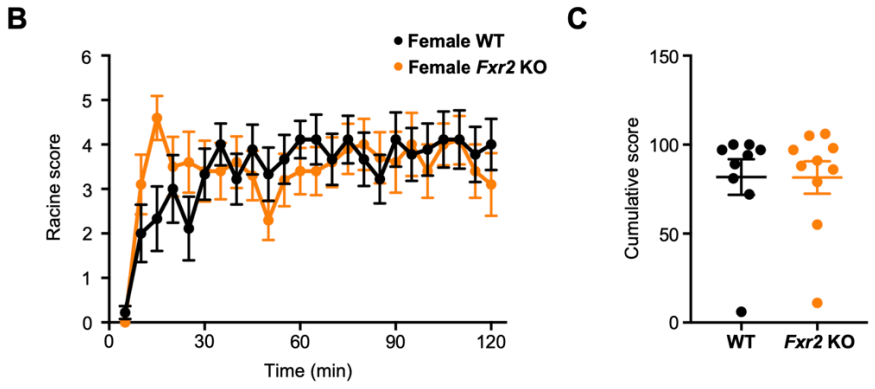


In line with this finding in humans, *Fxr2* KO female mice are not protected against prolonged kainic acid-induced seizures, in contrast to male *Fxr2* KO mice. We have added these data to the revised manuscript (*new Figure EV2B-C*). In the result section, we added the following text:

"Interestingly, female *Fxr2* KO mice did not display resistance to KA-induced seizures, coherent with the DECIPHER database (Fig EV2B-C). Therefore, we only considered males in all subsequent analyses."

In the discussion we added the following paragraph:

"While epilepsy and SE are more frequently observed in men (Hesdorffer et al., 1998; Coeytaux et al., 2000), mutations in the X-linked *PCDH19* gene lead to early-onset seizures specifically in women, leaving the male carrier largely unaffected (Dibbens et al., 2008). In this case, it is possibly due to tissue mosaicism of the X-chromosome in women (Dibbens et al., 2008). FXR2, instead, is not located on the X chromosome but some of its targets, such as *Hcfc1* and *Huwe1* (Fig 4B and Table EV4), are in both mouse and human. Further studies are required to address this gender-specific effect."



The description of the mouse model in the materials and methods section falls short. Three different genetics backgrounds are mentioned for the knockout, 129, B6 and FVB. Given the enormous difference in seizure susceptibility between these strains, please provide some more information on how the colony was maintained. I realize the colony was created with the 3

backgrounds, but as this was almost two decades ago, it makes a big difference how this one was maintained (by backcross with B6, FVB, other?). As for the wild type animals, where were these purchased and were these B6 or FVB.

We agree with the reviewer that different backgrounds can give rise to differences in seizure susceptibility. In all our experiments, we used WT littermates as control mice. To gather more precise information on the contribution of the background of the animals used in this manuscript, n = 6 brain tissues (from 3 WT and 3 *Fxr2* KO mice) were analysed by Charles River performing a genetic screening. The results arrived on November 2020 and confirmed that the background is >90% FVB congenic. We have now added this information in the Material and Methods section under the paragraph Animals.

Minor comments:

Introduction:
"...epilepsies, often referred to as epileptic encephalopathies."
This in not entirely true, epileptic encephalopathies refer to syndromic and severe forms of epilepsies, not to all.
We have revised the sentence as "During the last decade, next-generation sequencing techniques have led to the identification of many monogenic epilepsies and epileptic encephalopathies (Thomas and Berkovic, 2014; Zhou et al., 2018)."

"both individuals with Fragile X syndrome (FXS) and mouse models for FXS show increased seizure susceptibility"

Rather, individuals have an increased seizure frequency, whereas mice show increase susceptibility to audiogenic seizure induction.
This is a great suggestion, and we have revised the sentence as follows "Individuals with Fragile X syndrome (FXS) show increased seizure susceptibility and, likewise, mouse models for FXS display increased sensitivity to audiogenic induced seizures (Berry-Kravis, 2002; Curia et al., 2013; Sethna et al., 2017)."

"This difference can be attributed to elevated levels of ERK1/2 (extracellular kinase 1/2) phosphorylation and altered translation of specific mRNAs"
This has been postulated, but in my opinion not enough evidence has been provided in the papers referred to (that deal with human patient studies only) to substantiate this claim. Better mention as an hypothesis
We have revised the sentence as follows "This difference is thought to be due to elevated levels of ERK1/2 (extracellular kinase 1/2) phosphorylation (Curia et al., 2013; Sethna et al., 2017) and altered translation of specific mRNAs (Darnell et al., 2011; Fernández, Rajan and Bagni, 2013; Bagni and Zukin, 2019)."

Results:
1st par. I would suggest to add one/two sentences of explanation on the Open Targets Platform, as many readers may not be familiar with this platform and its potential.
We have added the following section to describe more extensively the Open Targets Platform: "In addition, we used the Open Targets Platform that provides an association index of a certain gene with different diseases (value 0 equals to no association, value 1 equals to substantial evidence)."

2nd par on the FXR2 knockout. The model is hardly introduced. What are the characteristics,

are homozygous or heterozygous knockouts used. What is known from this model? Is it known to display seizures? Has this been tested at all. In addition, The mice are generated from heterozygous intercrosses, were the full knockouts or the heterozygous knockouts used, or both.
The mice we used were full knockouts. We have now added a paragraph that describes the literature for the *Fxr2* KO mouse model as follows:
"We used the *Fxr2* KO model that displays several phenotypic traits similar to those observed in the *Fmr1* KO model, like altered synaptic plasticity, hyperactivity and impaired learning (Bontekoe et al., 2002; Spencer et al., 2006; Zhang et al., 2009). At the molecular level, a few studies in mice indicate that absence of FXR2P negatively affects the expression of hippocampal glutamatergic proteins, such as PSD-95 and GluA1 (Fernandez et al, 2015; Cavallaro et al., 2008; Guo et al., 2015). However, susceptibility to seizures has never been previously investigated in the *Fxr2* KO mice."

3rd par on transcriptome

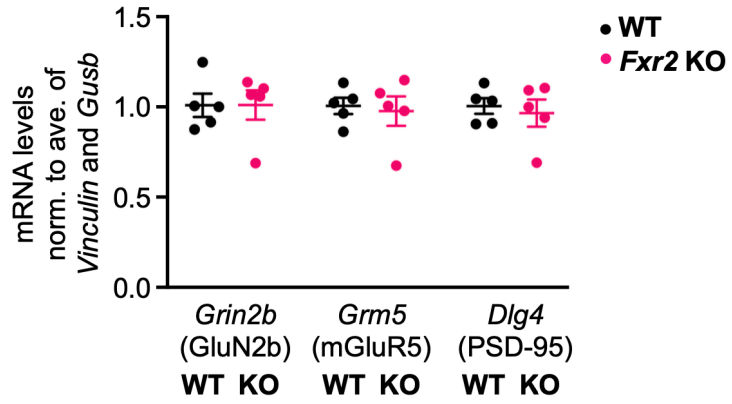
"Genes that were differentially expressed"

Rather, differentially bound by the FXR2P
We have revised the sentence as "we identified 488 genes that were differentially bound by FXR2P."

4th par on mRNA targets

Please mention GluK5 as a kainate receptor when first mentioning this in the text
We have added in parenthesis that GluK5 is a kainate receptor.

Discussion
"We therefore propose that FXR2P is responsible for the mRNA translation....."
Though the authors unequivocally demonstrated the involvement of FXR2P in the glutamatergic signaling, I do not find arguments in this work to narrow this down to the level of RNA translation
We understand the referee's correct point. To further test our hypothesis, we have analysed the basal levels of GluN2B, mGluR5 and PSD-95 mRNAs in hippocampus - performing qPCR - and did not find any difference between WT and *Fxr2* KO (see Figure below), while there is a difference at the protein level. Our findings further suggest that FXR2P is involved in the translation of these mRNAs. We have added this dataset in the revised manuscript (***new Figure 5B***). Consistently we have revised the discussion as follows: "Because the changes in GluN2B, mGluR5 and PSD-95 were only detected at the protein level and not at the mRNA level, it can be hypothesized that FXR2P is responsible for the translation of this group of mRNAs encoding proteins affecting the glutamatergic synapse."



Please include a few sentences on the outcome of the MEA recordings in the conclusion.
We have now incorporated a few additional sentences on the outcome of the MEA recordings that in the revised manuscript have been moved to the Supplementary data section (*highlighted in italic*).

"In conclusion, we provide evidence that *Fxr2* KO mice do not undergo long-lasting, and therefore potentially long-term harmful, SE. This phenotypic trait may depend on reduced excitability due to reduced hippocampal glutamatergic signalling. In fact, *Fxr2* KO hippocampi display reduced expression of glutamatergic components, including GluN2B and mGluR5. *Also, coherently with the in vivo data, we detected a decreased global excitatory network activity in the Fxr2 KO hippocampi ex vivo, using the multi-electrode array (MEA) that offers extracellular recording information on the network level with high spatial and temporal resolution.* Our results provide a molecular and cellular framework for a mechanistic interpretation of the susceptibility to prolonged SE and may also contribute to explain the epileptic phenotype reported in patients with the 17p13.1 deletion".

General:
Not sure that epilepsy can be named a "behavioral" outcome. Also I believe the name "behavioral epilepsy" is not widely accepted and I would suggest to avoid this term
We agree with the reviewer that epilepsy is a neurological disorder and that the term behavioural epilepsy is indeed incorrect. Therefore, we have removed the term "behaviour" and replaced it with "seizures".

Referee #2:

This study by Lo et al. investigated the roles of an RNA-binding protein FXR2P in neuronal excitability regulation and seizure susceptibility. The authors show that knocking out FXR2P leads to reduced seizure susceptibility presumably through ERK1/2 associated signaling. Strengths of this manuscript include good quality of the data, the use of proper controls and nice flow of the written document. However, while the data are properly presented and analyzed, the study is very preliminary. The main conclusion is built upon some superficial observation and is not supported by the data, which led to many overstatements. Many alternative mechanisms were not considered. Overall, the impact of this study is limited with its current data.

Major points:

1. The authors claimed multiple times throughout the manuscript that their data suggest the role of FXR2P in the development of status epilepticus (SE). This is not an appropriate statement. The data in this manuscript only indicate reduced excitability or seizure threshold in FXR2P knockout mice/neurons, which does not reflect SE in any way. Unless the author monitored chronic spontaneous seizures, it is inappropriate the suggest FXR2P affects SE.
We apologize for the lack of clarity in our statements. We may have generated confusion by inappropriately using the term "development" instead of "duration" or "maintenance". We have now replaced the term "development" with "duration" or "maintenance".

About our statement that FXR2P affects SE, we based our findings/conclusions on the definition given by the International League against Epilepsy (ILAE). ILAE defines SE as

"*a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally, prolonged seizures (after time point t1). It is a condition, which can have long-term consequences (after time point t2), including neuronal death, neuronal injury, and alteration of neuronal networks"* (Trinka et al., Epilepsia, 2015).

Therefore based on this definition, chronic epilepsy that is characterized by the occurrence of spontaneous recurrent seizures is a possible long-term consequence of SE. Long-term consequences after t2 were not within the scope of this manuscript.

In preclinical research, SE is commonly investigated using a methodology similar to what we used in this study, i.e. by treating mice or rats with chemoconvulsants like kainic acid or pilocarpine and observing the animals for several hours, monitoring motor seizures.

Similar to other authors, we defined SE as occurrence of longer than 5 min uninterrupted motor seizures (Racine stage 3 or above). Both genotypes developed motor seizures of Racine stage 3 or above, suggesting similar propensity to develop seizures. However, while these seizures persisted in the WT and developed into motor SE, the maintenance of such severe seizures lasted much less in *Fxr2* KO mice.

We added the following text to include the current definition of SE:
"The International League Against Epilepsy defines SE as "a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally, prolonged seizures[…]. It is a condition, which can have long-term consequences[…], including neuronal death, neuronal injury, and alteration of neuronal networks." (Trinka et al., 2015).

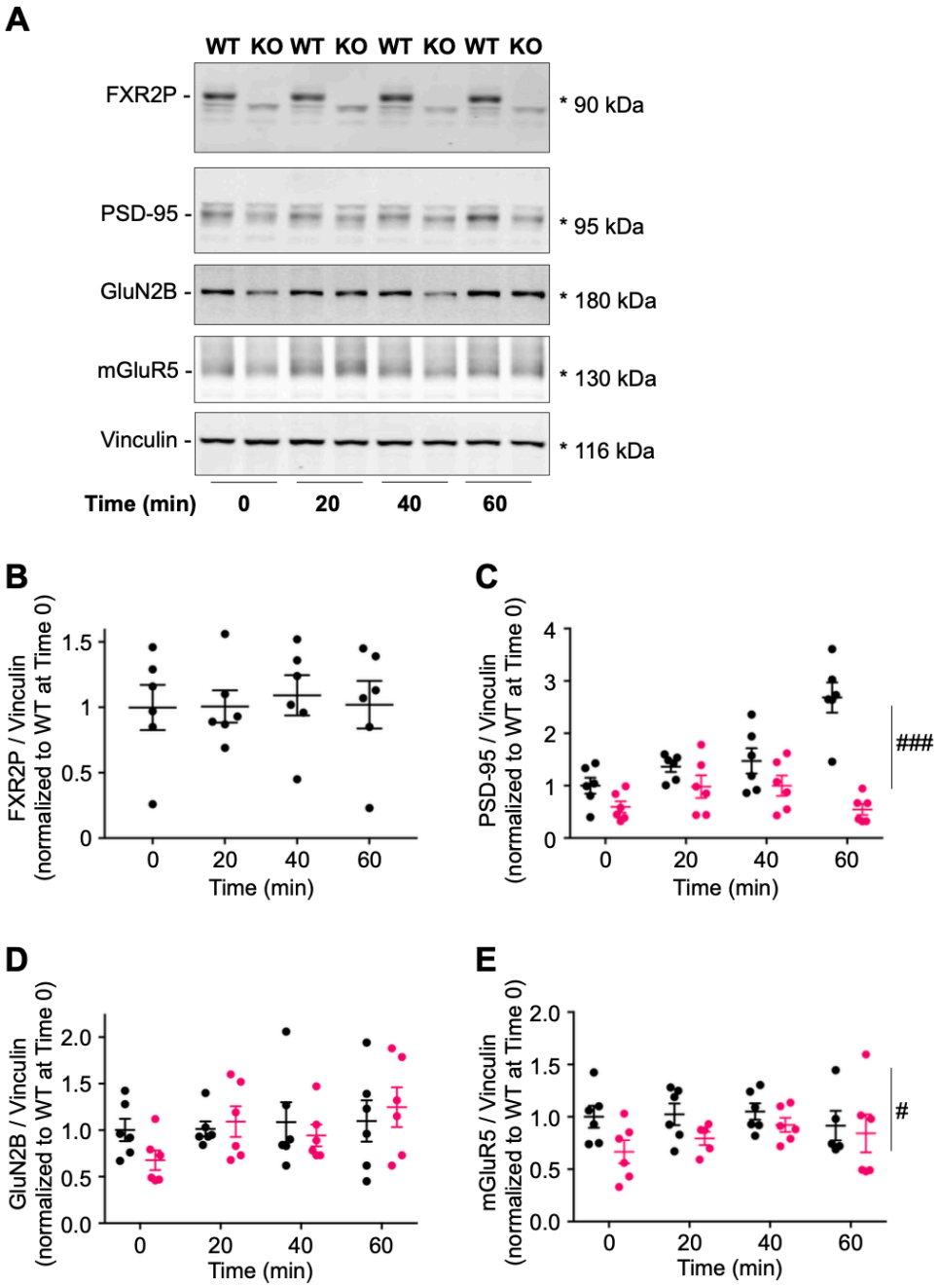
Both WT and *Fxr2* KO mice developed motor seizures of Racine stage 3 or above, suggesting similar propensity to develop seizures. However, while motor seizures persisted and developed into motor SE in the WT, SE does not occur in the *Fxr2* KO mice.
KA acts through the kainate receptors (Falcón-Moya, Sihra and Rodríguez-Moreno, 2018), and we found that the only kainate receptor among the FXR2P targets, *GluK5*, was unchanged in WT and *Fxr2* KO hippocampi, further substantiating that seizure initiation is not affected".

2. The authors showed that ERK1/2 signaling is altered in FXR2P KO mice and suggest that this is the underlying mechanism following GluK5 activation and altered seizure activity. Many factors can cause activation of ERK1/2. Without experiments to evaluate GluK5, how can the authors conclude that the effect is through GluK5 as suggested in Fig. 5D? The effect could very likely be a secondary effect following KA-induced hyperactivity in the brain.
We agree with the reviewer that ERK1/2 can be activated by many factors. However, we do not postulate that ERK1/2 is the underlying mechanism, but it is a reflection of the hippocampal state. ERK1/2 activation as well as c-fos are regularly used in epilepsy and seizure experiments to reflect the hippocampal state. Since both *Fxr2* KO and WT mice are able to show seizures in the initial phase of the experiment, we do not expect that the difference lies between GluK5 and ERK1/2, but more with the glutamatergic proteins required to maintain the seizures in a later phase.
We agree with the reviewer that KA action not necessarily goes only through the subunit GluK5, and have revised the model (*Figure 5D, now Figure 7*). We summarize that KA activates the different types of GluK receptors and also added the reference Falcon-Moya et al., Front Mol Neurosci, 2018 in the discussion.

3. What is the mortality rate in the seizure experiments? Pilocarpine is especially known to elicit high mortality. The authors should provide information about the mortality in their experiments.
The referee is correct and indeed a mortality rate has been documented in animal experiments with seizure. To avoid the mortality of the animals during the experiment as much as possible, we have included a humane endpoint – in agreement with the approved license for animal experimentation. Specifically, when a mouse experiences 15 consecutive minutes of Racine score 3 or above, then the experiment is prematurely terminated, the mouse euthanized and excluded from any further analysis. As described in the Material and Methods section, this occurred for 2 *Fxr2* KO and 3 WT mice. We have now described this procedure more extensively:
"To avoid the mortality of the animals during the experiment as much as possible, we have included a humane endpoint – in agreement with the approved license for animal experimentation. Specifically, when a mouse experiences 15 consecutive minutes of Racine score 3 or above, the experiment is prematurely terminated, the mouse euthanized and excluded from any further analyses (*Fxr2* KO n=2, WT n=3)."

4. The authors' model suggests that FXR2P's function primarily occurs later during the seizure progression. Does the expression of FXR2P's targets (mGluR5, PSD-95 etc.) truly follow the same time course as ERK1/2 activation after KA injection (Fig. 4D) and, if yes, does that depend on FXR2P? Does FXR2P expression change over the course of seizure progression?
We agree with the reviewer that it would be interesting to track the development of the different targets and FXR2P over time. Following the reviewer's suggestion, we have performed some additional experiments (see Figure below). The data are now integrated in the revised manuscript.

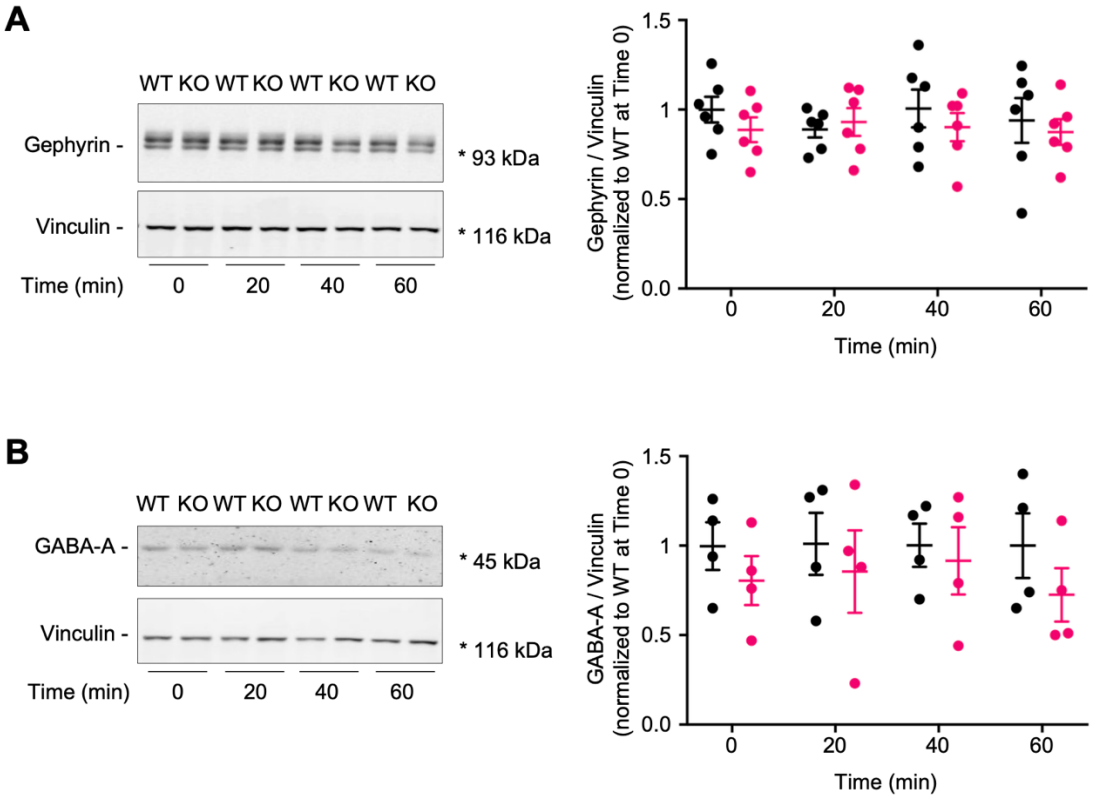
“Finally, we investigated if the expression of FXR2P and of a few targets involved in the glutamatergic signalling change over time (*Fig EV4A*). At first, we noticed that FXR2P expression in WT hippocampi did not change over time and was not affected by KA stimulation (*Fig EV4B*). The three glutamatergic FXR2P targets, namely PSD-95, GluN2B and mGluR5 showed different expression patterns. Specifically, PSD-95 expression slightly increased over time in WT and remained reduced and unchanged in *Fxr2* KO hippocampi (*Fig EV4C*). Second, GluN2B expression did not change in hippocampi upon 60 min KA stimulation (*Fig EV4D*). Finally, mGluR5 expression did not change over time upon stimulation in both genetic condition, but was overall consistently lower in *Fxr2* KO hippocampi (*Fig EV4E*). Altogether in *Fxr2* KO hippocampi, the expression of GluN2B is restored upon 60 min stimulation, while PSD-95 and mGluR5 levels remained reduced compared to WT hippocampi.”



5. The authors nicely showed reduced seizure activity in FXR2P KO mice. However, the MEA data in Fig. 5 does not provide much beyond that. It is unclear why Picrotoxin had to be added to increase neuronal firing. Can the author rule out any possibility that FXR2P KO and WT mice respond differently to Picrotoxin? Would it be possible that GABAergic signaling is altered in FXR2P KO mice that led to the observation in Fig. 5A? If the authors really need stimulation, why not use KA?

We understand the referee's point. Indeed Figure 5 was meant to extend the *in vivo* observations using an *ex vivo* approach. We focused on the glutamatergic component and used

a protocol to induce epileptiform bursts in an *ex vivo* set up (Gong et al, PLoS ONE, 2014; Gafurov and Bausch, J Neurophys, 2013). The reviewer is correct that, at this stage, we cannot rule out an altered GABA signaling in *Fxr2* KO mice. However, this was not within the scope of the current manuscript and will be addressed in a future study - under more favorable experimental circumstances. Due to COVID we had to largely decrease our mouse colonies.
To at least partially address the reviewer's question, we analyzed the protein expression of GABA-A receptor and Gephyrin, an anchor protein for postsynaptic inhibitory receptors, in the hippocampus (same extracts tested for the glutamatergic targets). While Gephyrin levels did not change between WT and *Fxr2* KO hippocampi, a tendency to lower expression of GABA-A receptor was detected in *Fxr2* KO hippocampi compared to WT (*Figure 1 for the reviewer only*). Nevertheless, further investigations are needed to make a clear claim about GABA-A receptor expression. Because the downregulation of the glutamatergic components was clearly detected at the level of total hippocampi (contrary to the GABA-A levels showing only a tendency), we think that the reduction in SE maintenance is mainly due to the excitatory component.



6. Related to point #4, it would be more informative if the authors can compare basal activity and after KA stimulation. This would tell us whether the effect is truly associated with KA or the neurons are basally more excited to begin with. It would also be better if a time course as in Fig. 4 can be applied. MEA is a power tool. It is a pity that the authors did not take advantages of this system to obtain more information.

7. Since FXR2P seems to regulate the expression of many regulators associated with glutamatergic signaling (Fig. 3), it would be useful if they authors can also evaluate synaptic transmission rather than only MEA recording.

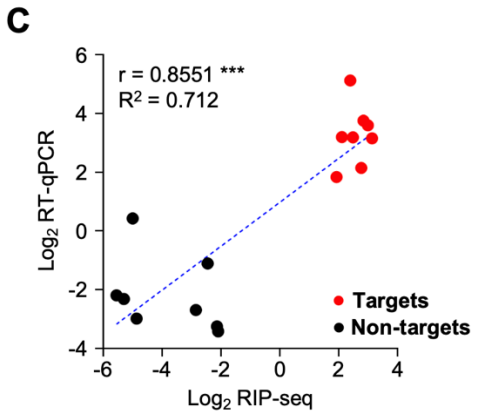
We agree with the reviewer that the investigation of the neuronal activity before and after KA stimulation would be highly informative. Our current findings highlight that at the basal level, *Fxr2* KO hippocampal slices are less excited, and at a disadvantage compared to WT slices. This however would not necessarily mean that upon KA they remain low in burst activity, or could potentially get to the level of WT excitability. Unfortunately, this experiment would require considerable time to execute and analyse and due to the current situation: COVID-19, we are not in the position to perform it.

Referee #3:

In the present manuscript, Bagni and colleagues investigate the role of the RBP FXR2P, a member of the family of Fragile-X-related proteins, in epilepsy. They report that the genetic loss of FXRP2 in mice reduces specifically the responsiveness to kainate, but not pilocarpine-induced epileptic seizures. By RIP-seq, they identify hundreds of mRNAs that are bound by FXR2P in the mouse brain, in particular those associated with glutamatergic synaptic transmission. Downregulation of these components in FXR2P ko mice correlates with the inability to sustain ERK activation after kainate. FXR2P ko slices show reduced burst activity compared to wt slices in response to picrotoxin. The authors propose a model whereby FXR2P stabilizes important components of glutamatergic synapses, thereby gating seizure activity after excessive stimulation of glutamatergic signalling.

Overall, this is a well conceptualized study that provides novel insight regarding the role of FXR2P in the regulation of epileptic seizures. Before these results can be published, the authors should revise their manuscript according to my comments below.

Specific comments:
1. The RIP-seq experiments needs more validation. An independent RNA-IP followed by qPCR for some candidates should be performed. In general, it was unclear why the p-value for the differential analysis between WT and KO was set to 0.1. According to the gene lists provided, an adjusted p-value of 0.05 would have already yielded >400 genes.
Following the reviewer's suggestion, we have added a validation that shows a significant positive correlation between RIP-seq values and RT-qPCR values in 8 epilepsy-relevant FXR2P mRNA targets and 8 FXR2P mRNA non-targets (*new panel Figure 4C* and see Figure below).

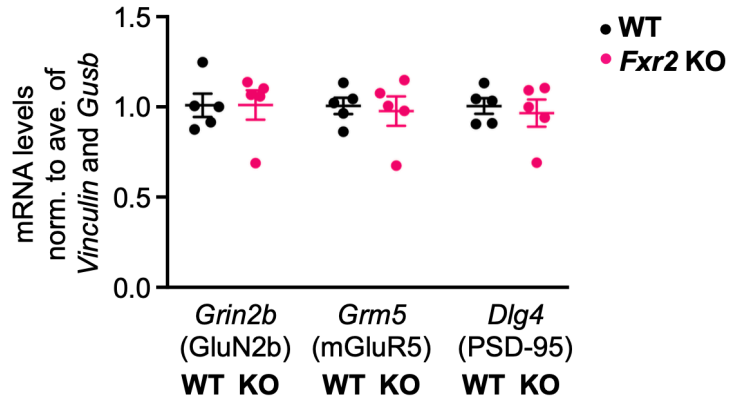


The reviewer is correct with the remark "According to the gene lists provided, an adjusted p-value of 0.05 would have already yielded >400 genes". We are using an adjusted p-value (FDR) of 0.1 and not the conventional p-value cut-off. This adjusted p-value (FDR) of 0.1 is conventionally used in the field for multiple testing correction and is also recommended by the authors of the Deseq2 R package. Indeed, the adjusted p-value (FDR) implemented in Deseq2 uses the Benjamini-Hochberg (BH) adjustment (Benjamini and Hochberg, 1995). This method calculates for each gene an adjusted p-value that answers the following question: "if one called significant all genes with an adjusted p-value less than or equal to this gene's adjusted p value threshold, what would be the fraction of false positives (the false discovery rate, FDR) among them". In other words, among all the significant genes we identified (488 genes) we tolerate a 10% false discovery rate (49 genes). In addition, all the 488 genes we identified have a

significant conventional p-value lower than 0.05. We clarified this in the method section and the *revised Table EV1* shows both the (unadjusted) p-value and adjusted p-value (FDR).

2. Some more detailed description of the GO-term analysis is required. For example, what was the background gene list used? Using results from the KO appears problematic, since the genes recovered from those IPs only represent non-specific binders. A better background would be the complete list of genes which are actually expressed in the system (e.g. determined by standard polyA RNA-seq).
Following the referee's comment we have clarified this important point in the Material and Methods section. For the gene ontology analysis background we have used all the genes corresponding to 14'809 genes (with a minimum of 30 reads detected in both WT and KO).

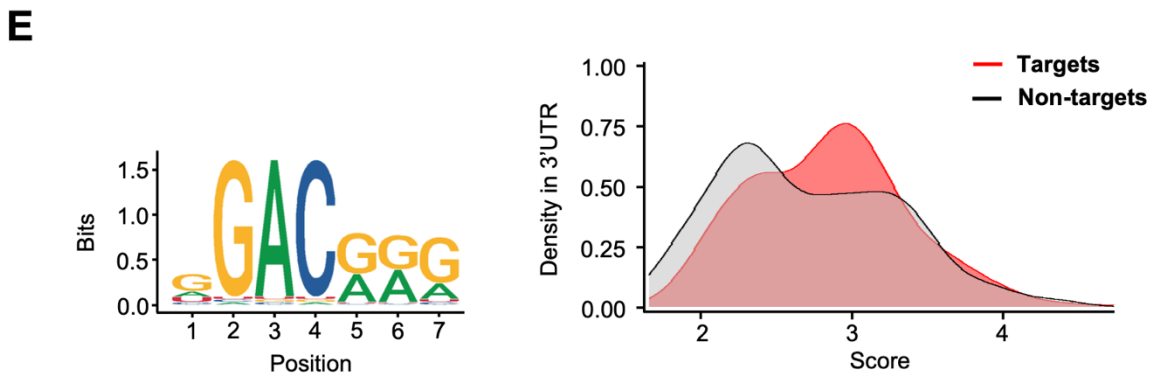
3. From the few candidates that were followed-up, it appears that all of them are downregulated at the protein level in the absence of FXR2P. Is this also true for the respective mRNAs? Does FXR2P in general stabilize target mRNAs, or is the regulation rather happening at the level of mRNA translation? Is there an enrichment of binding motifs for FXR2P in the RIP-targets, e.g. in the 3'UTR? Some additional mechanistic insight regarding the function of FXR2P in target gene regulation would be desirable.
Following the reviewer's suggestions we have investigated the mRNA levels for the respective glutamatergic targets and they do not change (*new Figure 5B*). This dataset supports the hypothesis that FXR2P regulates glutamatergic mRNA translation.



Concerning the possible binding motifs for FXR2P, we made use of the RBPmap (http://rbpmap.technion.ac.il) that maps the predicted binding motifs on the respective 3'UTRs and identified a possible binding region. We have added the outcome of the analysis in the Results section (*new Figure 3E*, see Figure below) and text as follows;

"To identify possible binding sites of FXR2P, we analysed the occurrence of the putative FXR2P-binding sequences (Ray et al., 2013) in the FXR2P transcriptome (Fig 3C, 3'UTR of the 488 targets) using the RBPmap tool (http://rbpmap.technion.ac.il; (Paz et al., 2014)). In accordance with its highly degenerate nature ((Ray et al., 2013); Figure 3E, left panel), the FXR2P-binding motif is frequently found in the 3'UTRs of FXR2P target as well as non-target mRNAs. Of note, higher-scoring FXR2P-binding sequences are overrepresented in target 3'UTRs with respect to an equally-sized set of non-target 3'UTRs (Figure 3E, right panel). A higher score corresponds to a stronger binding of FXR2P to the respective sequence,

suggesting that the putative consensus sequence might contribute to the recognition of FXR2P targets. As for other RNA binding proteins, it can be hypothesized that the sequence domain of FXR2 is furnished/surrounded by additional cis-acting elements that are recognised by other, cooperating RNA-binding proteins (Achsel and Bagni, 2016). Additional studies are required to conclusively identify bona-fide FXR2 recognition domain/s."



4. The overlap between epilepsy genes and FXR2P targets appears substantial, but is this overlap really more than expected by chance? What statistical test was used to draw this conclusion?
We investigated the hypergeometric probability distribution with Fisher's exact test to verify whether the overlap is more than expected by chance. The statistical test is added in the Material and Methods as well as in the figure legend.

5. Why were the number of slices different for WT and KO in the MEA recordings? It would also be important to report the number of individual animals these slices were derived from.
This information was provided in the Material and Methods, we have written it more clearly, and also added this information in the figure legend. Briefly, 9 slices were taken from 6 WT mice, and 16 slices were taken from 8 *Fxr2* KO mice. The number of mice comes from the same litters and therefore the difference is based in the genotype we obtained.

6. What is the effect of FXR2P knockout on basal glutamatergic transmission? This could be for addressed by patch-clamp electrophysiological recordings of mEPSCs in hippocampal slices.
We thank the referee for this comment, and it is similar to what referee #2 asked (question #7). As stated there, this experiment would require considerable time to execute and analyse (in addition to the limiting current situation: COVID-19), and would exceed the duration of the revision period.

1st Revision - Editorial Decision 18th Jan 2021

Dear Claudia,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees that were asked to assess it.

As you will see, referee 2 is not satisfied with the revisions. I asked referee 3 for cross-comments and s/he feels that referee 2's concerns are sufficiently well addressed. Please see the cross-comments below and please address point 1 in the manuscript text - especially the title and abstract need to be rewritten - so that we can proceed with the official acceptance of your manuscript.

A few other editorial changes will also be required:

- The statement in the data availability section should read: No data generated by this study were deposited in public databases.

- Please fill in the last section of the author checklist, the dual use research of concern. You need to state whether your data could be used for research of concern.

- Please correct the reference style, up to 10 authors need to be listed before "et al".

- Fig. 7 panels are not called out. Fig. EV3 panels are not called out. Please correct.

- Tables EV1-4 should be called Datasets EV1-4 and need to be uploaded as individual files, as all EV figures and all EV tables need to be uploaded as individual files. Tables EV5 and 6 should be called Tables EV1 and EV2. All tables need to have a title and/or legend in the first tab of the excel file. Please double-check that all callouts are corrected in the manuscript text. The EV figure legends should be added to the manuscript file after the main figure legends.

- The Funding info should be included in the Acknowledgements.

- The Figure Legends should be moved to after the Reference list.

- I attach to this email a related manuscript file with comments by our data editors. Please address all comments in the final manuscript.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have any questions.

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have carefully responded to the questions addressed by the referees and the manuscript has much improved.

Referee #2:

As I mentioned in my initial review, the manuscript is nicely written but somewhat preliminary with overstatements. After the revision, the authors refused to do most of the experiments I requested, citing that those experiments are either out of their scope or too time consuming. It is understandable that, given the COVID situation, it is difficult to conduct complex experiments. However, without some critical data and with only toning down the statements, in my opinion the manuscript is further away from the quality that is needed for EMBO Reports.

Referee #3:

In the revised manuscript, the authors have satisfactorily addressed all my previous concerns. I therefore recommend publication.

Cross-comments by referee 3 on referee 2's concerns:

1. I am not an expert in epilepsy research, but from my knowledge would also think that the experiments do not really address "status epilepticus", which usually involves the occurrence of spontaneous seizures over the long-term (days to weeks). To get at this, technically more challenging experiments, like long-term EEG recordings in behaving mice (using implanted devices) would be required. I didn't feel that such experiments would be absolutely required for this ms, but the authors could at least be asked to tone down their statements.
2. I would agree with the author's response that they never claimed that ERK activation was the underlying mechanism. The data on ERK is in my opinion convincing, but of course correlative.
3. I think they addressed this point in the rebuttal letter.
4. The authors provided new data regarding this point. At least the time course of PSD-95 (and to some extent mGluR5) followed the ERK activation, so I don't have a major issue here.
5. The MEA data is ok, but it would of course have been nice if the authors also included patch-clamp electrophysiological recordings. I wouldn't however see this as a Conditio-sine-qua-non.
6. Again, i would see KA induction experiments as nice add-on, but not as absolutely mandatory at this stage.
7. See point 5.

Point by Point to the cross-comments by referee 3 on referee 2's concerns.

1) We have changed the title from "**Absence of the RNA binding protein FXR2P protects against status epilepticus"** into "***Absence of the RNA binding protein FXR2P prevents the prolonged phase of kainate-induced seizures*".**
2) We have revised the abstract adding the following sentence: Taken together, these findings suggest that the lack of FXR2P reduces the abundance of glutamatergic proteins, which may be required for the transition to seizures that self-sustain for up to 2 hours, *i.e.*, SE.
3) WE have added a sentence in the Discussion: In conclusion, we provide evidence that *Fxr2* KO mice do not undergo long-lasting motor seizures. Even though we did not look at long-term consequences, we expect that the *Fxr2* KO mice do not develop epilepsy, *i.e.*, increased propensity to spontaneously seize long after the insult, which is normally observed after kainate-induced SE (Lähteinen *et al*, 2002).

   The reviewers' point arises from a different definition of "status epilepticus" and "epilepsy". Because the correct usage of terminology is very important in science, we again scrutinized the definition of these terms. **Status epilepticus** is commonly defined as a seizure with 5 minutes or more of continuous clinical and/or electrographic seizure activity or recurrent seizure activity without recovery between seizures (see for example Lowenstein *et al.*, Epilepsia, 1999; Brophy *et al.*, Practice Guideline, 2012; Al-Mufti *et al.*, Critical Care Clinics, 2014). **Epilepsy**, in contrast, is a disease characterized by an enduring predisposition to generate epileptic seizures (see for example Fisher *et al.*, Epilepsia, 2014).

   It could be that reviewer #2 uses "status epilepticus" in the meaning of "epilepsy" as defined above, and therefore asks for evidence of enduring propensity to generate seizures. Here we are interested in the duration of the initial set of seizures, if they continue without intermission (WT) or decrease (*Fxr2* KO), *i.e.*, if the status epilepticus is reached or not.

   We would like to maintain our definition of status epilepticus as we explained in the previous point by point, see also the first sentence of the current Abstract and Discussion. To summarize, to avoid confusion, we now have 1) substituted the word "status epilepticus" in the title with "prevents prolonged phase of seizures". We also tuned down the title by stating that "kainate-induced" seizures are affected (in fact, pilocarpine-induced seizures are not affected). 2) added a sentence at the end of the abstract where the period

of our interest is defined as the first 2 hours after kainate induction. 3) added a sentence at of the discussion where we explicitly state that we did not investigate the long-term effects of SE.

4) We have added the following sentence: DATA AVAILABILITY. No data generated by this study were deposited in public databases and are available upon request from the corresponding authors.
5) We have modified Fig 7 (model seen from 2 different angles) and used part of it (panel B) to make a graphical abstract.

2nd Revision - Editorial Decision 29th Jan 2021

Prof. Claudia Bagni
Department of Fundamental Neuroscience
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Switzerland

Dear Claudia,

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

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Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: CLAUDIA BAGNI
Journal Submitted to: EMBO Reports
Manuscript Number: EMBO R-2020-51404V1

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For behavioral experiments a maximum of 15 animals per group gave an adequate power for statistical analyses. For Western blot analysis a maximum of 10 samples per group gave an adequate statistical power. For the electrophysiology experiment a maximum of 15 slices gave an adequate statistical power.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For both the behavioral and electrophysiology studies, sample size estimations were performed with a power of 80%, an alpha of 0.05 and an effect size of 0.5 SD (Cohen's d). For Western blot analyses, sample size estimation was performed with a power of 80%, alpha of 0.05 and an effect size of 1.4 SD.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Exclusion criteria for seizure experiments were pre-defined and detailed in the Material and Methods (based on 15 min consecutive duration of Racine score >= 3). For Western blot analysis, values that are not within the 2.5 SD range from the mean were considered as outliers and excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	The experimenter was blind to the compound (i.e., vehicle or convulsant) injected to the animals and to the genotype. Vehicle and convulsant tubes were indicated as 1 or 2. The scoring of the seizures was likewise performed blind.
For animal studies, include a statement about randomization even if no randomization was used.	Animals were randomly assigned to either vehicle or treatment condition.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The results were processed in a blind-manner, i.e., no information concerning genotype and treatment were available during the data processing. Upon data process completion, information on genetype and treatment were added to the final analyses.
4.b. For animal studies, include a statement about blinding even if no blinding was done	The scoring was performed by one co-author blind to the genotype and treatment.
5. For every figure, are statistical tests justified as appropriate?	Yes- the detailed statistical analyses are reported in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	All the generated data met the assumptions of the tests. Shapiro-Wilk test showed no difference from the normal distribution.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	The variance between groups were statistically similar in all the cases.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	For the antibodies, we have provided a Table (EV5) containing the protein name, company name, the catalogue number, the molecular weight and the concentration used.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The Fxr2 mouse model, strain, gender, age and housing conditions are detailed in the Material and Methods. We used an in-house mouse model, that was kindly provided by Dr Rob Willemsen. Notably, WT and Fxr2<tm1Cgr> mice were >90% FVB congenic. Male mice were used throughout the study, except in one dataset (Fig EV2C-D, in this case they were females). Mice were group-housed per 3-5 in polyethylene cages (16 cm x 28 cm x 11 cm; 448 cm2 living space) and kept in humity and temperature-controlled environment.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The experiments were approved under the animal license VD3151.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance. We have reported every relevant aspect of the animal studies in the Material and Methods.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The RNA seq data is provided as Table EV1.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Currently all the data are safely backed up and filed on our Institutional server (University of Lausanne) that is not accessible to external parties. In addition the data are stored on personal computers and documented in lab books. The data can be therefore easily retrieved and made available to the entire scientific community upon request.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	The compounds used in this study are not found in the List of Select Agents.