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A microRNA-129-5p/Rbfox crosstalk coordinates homeostatic downscaling of excitatory synapses

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Transaction Report:

(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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 02 November 2016

Thanks for submitting your manuscript to The EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see the referees find the analysis interesting but also that the further experiments are needed to fully support the conclusions. I would therefore like to invite you to submit a revised version that takes into consideration the raised concerns. The issues raised are clearly outlined below and I will not repeat them here. Let me know if we need to discuss any of them further

It is EMBO Journal policy to allow a single major round of revision and that it is therefore important to address the raised concerns at this stage.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you

foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

In their study, Rajman et al. investigate the molecular mechanisms of homeostatic downscaling of excitatory synapses. This regulatory negative feedback permits neurons to reduce firing rates in response to chronically elevated network activity. In particular, they screened miRNA expression in hippocampal neurons. Combining small RNA-seq, bioinformatic target predictions, qRT-PCR and Western-blot, they identified one miRNA, miR129-5p and two target mRNAs, Atp2b4 and Dcx, as a molecular pathway involved in homeostatic downscaling. In addition, they show a cross-talk between Rbfox1, a RNA-binding protein, and miR-129-5p. In control conditions, Rbfox-1 binds to Atp2b4 and Dcx 3'UTRs to promote their translation. In contrast, in picrotoxin-treated neurons, Rbfox-1 expression is down-regulated by miR-129-5p whereby allowing the repression of Atp2b4 and Dcx.

This study is of particular interest since the mechanisms of excitatory synapse downscaling are involved in the main brain functions, both in physiological and pathological conditions. This study includes a very large set of experiments and data are supporting most of the conclusions. However, a few points need to be clarified.

Major points:
- My main point concerns the treatment used by the authors to induce homeostatic downscaling. Both the picrotoxin (PTX) and the bicuculline (Bic) treatments have been well characterized in previous studies. Thus, it is not clear why part of the work has been performed with PTX and the other part with Bic. Authors say that "electrophysiological recordings were more stable with Bic compared to PTX", this is quite unexpected and suggests that PTX and Bic treatments may not lead to the same molecular regulation. The authors claim that gene activation is the same for both treatments based on the analysis of 4 genes (Atp2b4, Camk2, GluA1 and Dcx), this is insufficient if you consider that PTX treatment significantly affects the expression of 957 genes (Fig. 4A and suppl. Tab. 3). Electrophysiological recordings of PTX-treated neurons should be presented to fully confirm the involvement of miR-129-5p and Atp2b4 in downscaling mechanisms.
- The authors indicate that miR129-5p has been previously identified as a regulator of potassium channel Kv1.1 expression (Sosanya et al. 2013) but they do not show their own results. Looking at their RNA-Seq data, it shows an up-regulation of Kv1.1 after PTX treatment. This result is opposite to the expected regulation in this condition where miR-129-5p is up-regulated. Authors should comment on that.
-Authors investigated target mRNAs of miR-129-5p with a nice set of experiments. Their demonstration that miR-129-5p is targeting Atp2b4 is really convincing. In contrast, Dcx targeting is less obvious. In particular, the luciferase experiment shows a regulation of Dcx-reporter even when miR-129-5p site is mutated (Fig. 5D) and the associated statistic is, in my opinion, not well performed. Indeed, authors have to compare the results obtained with WT reporter and mutated reporter, but they did two separate one-sample t-tests for WT and mutated reporters. Since this kind of test is less stringent, authors should confirm that result by directly comparing the two conditions with a t-test (or a non-parametric test if distribution is not normal).
-To summarize their findings, authors did a scheme in Fig. 8G that suggests a regulation of Pp3ca, Rbmx and Celf1 by miR-129, miR-212 and miR-543. However, their data do not support this regulation pathway. A full demonstration, using at luciferase reporter and/or over-expression of candidate miRNAs, should be performed before drawing this conclusion.

Minor points:
-In the introduction: homeostasis is not only involved in development and injuries but also in learning mechanisms in physiological conditions.
-It is not clear how authors did miRNA screening in PTX model, is this based on classical RNA

extraction or on Ago2-IP? Please, clarify.
-It is not clear how authors selected PTX-downregulated genes from RNA-Seq data to perform qRT-PCR.
-An interesting finding is the anti-correlation of miR-129-5p and Atp2b4 in human TLE patients. Since authors try to demonstrate that Dcx is also a target of miR-129-5p, it would be interesting to quantify Dcx levels as well.

Referee #2:

This manuscript describes experiments designed to assess the role of microRNAs in the homeostatic down scaling of excitatory synapses and epilepsy. Using a classic model of synaptic down scaling which uses the GABAR antagonist picrotoxin to increase neuronal excitability, the authors nicely show that a number of microRNAs including miR-129-5p are up regulated following this treatment. They also provide evidence that miR-129-5p is required not only for synaptic down scaling in vitro but for the induction of epileptic seizures in vivo. Probing into the mechanisms, the authors also identified miR-129-5p target sequences in the 3'UTRs of a variety of transcripts encoding proteins that are down regulated by picrotoxin including Atp2b4 and Dcx. Consistently, they found that miR-129-5p overexpression suppressed the expression of these proteins and that mutations in these target sequences, suppressed the inhibition of a luciferase reporter by picrotoxin or miR-129-5p. Interestingly the over-expression of Atp2b4 or Dcx partially prevented synaptic down scaling supporting evidence by others that they play important roles in the homeostatic process. The authors expanded on this core observation to show that the RNA binding protein, Rbfox1, functions in a complementary manner with miR-129-5p to regulate mRNA translation and stability. Specifically, they found that Rbfox1 positively regulates mRNA translation and stability by binding the 3'UTRs of several mRNAs associated with homeostatic synaptic scaling. Intriguingly the 3'UTR of Rbfox1 mRNA also contains miR-129-5p target sequences and is regulated by miR-129-5p. As such during a picrotoxin treatment, miR-129-5p not only help silence specific target transcripts but also those such as Rbfox1 that normally stabilizes mRNAs for translation, causing a yet further suppression of key synaptic proteins.

Overall this is a comprehensive study that combines a variety of biochemical, functional and proteomic approaches to provide mechanistic insights into how changes in synaptic activity can modulate the translation of transcripts associated with these homeostatic mechanisms. Importantly the data presented appear to be rigorous and of high quality. Nonetheless, there are a few outstanding issues that require some attention.

1. The discussion is very high level and does not comment or expand on the data collected.
2. Two examples are presented in figure 2. The authors claim that miR-129-5p over expression suppresses PTX dependent down scaling of dendritic spine as well as mEPSC amplitude, yet it is clear from the images and such that miR-129-5p over-expression alone does this, making it difficult to conclude that miR-129-5p has specifically block PTX dependent down scaling or something more general. As such the claim is not supported by the data.
3. It is unclear why the authors change their seizure inducing models. They make a big deal about using electrical stimulation but then switch to a kainic acid model for all other assay. Some explanation is required. It is also unclear why the authors think that PBS injection is a proper control for their Anti-miR-129 injections. Seems that a scrambled or mutant oligo would be more appropriate.
4. The images in figure 6 have the same problem as the authors do not address the over-expression effects of Atp2b4 and Dcx on spine number and mEPSC amplitudes in control treated cells. Also the data in 6A and 6B are duplicated and should be merged into one panel.
5. On page 19 in the discussion the authors bring up a new topic not found in the results, namely that there is a global reduction of the neuronal calcium extrusion system. If this is such an important point to make, the some reference in the results should be made.

1st Revision - authors' response 28 April 2017

Referee #1:

Major points:

Ref#1: My main point concerns the treatment used by the authors to induce homeostatic downscaling. Both the picrotoxin (PTX) and the bicuculline (Bic) treatments have been well characterized in previous studies. Thus, it is not clear why part of the work has been performed with PTX and the other part with Bic. Authors say that "electrophysiological recordings were more stable with Bic compared to PTX", this is quite unexpected and suggests that PTX and Bic treatments may not lead to the same molecular regulation. The authors claim that gene activation is the same for both treatments based on the analysis of 4 genes (Atp2b4, Camk2, GluA1 and Dcx), this is insufficient if you consider that PTX treatment significantly affects the expression of 957 genes (Fig. 4A and suppl. Tab. 3). Electrophysiological recordings of PTX-treated neurons should be presented to fully confirm the involvement of miR-129-5p and Atp2b4 in downscaling mechanisms.
Our response: We fully agree that a consistent use of one of the drugs would have been desirable. Based on our previous experience (Fiore et al., 2014), we focused on pictrotoxin, and obtained very reliable results in proteomics, RNAseq, biochemistry and morphological analysis. However, repeated attempts by our electrophysiology postdoc at that time, Dr. Ayla Aksoy-Aksel, to reproduce the reported PTX-mediated downscaling of mEPSC amplitudes by patch-clamp recordings of dissociated hippocampal neurons failed for unknown reasons. In contrast, bicuculline (Bic)-treated neurons displayed a robust and reproducible downscaling of mEPSC amplitudes (Fig. 2B, 6B). We would have been willing to further optimize PTX recordings in the context of this revision, but unfortunately Dr. Aksoy-Aksel left the lab end of 2015, so that we do not have any scientist with patch-clamp experience in the institute anymore. We therefore considered alternative molecular biology and cell biology assays to demonstrate that PTX and Bic effects on multiple parameters are highly comparable. In addition to Western blot data for five genes (suppl. Fig. 1F), we now additionally present full transcriptome analysis of Bic-treated neurons by RNAseq (suppl. Fig. 1E). We found that gene expression changes induced by PTX or BIC were highly correlated ($R=0.731$; $p<0.0001$), providing strong support that the two drugs have very similar effects on neuronal gene expression. Furthermore, we have now repeated miR-129-5p inhibition and Atp2b4 overexpression in the context of Bic treatment (suppl. Fig. 1D), and found that both had very similar effects on dendritic spine size in Bic- compared to PTX-treated neurons. Taken together, we are highly confident that the two GABA-A receptor blockers PTX and Bic elicit highly similar effects on gene expression and neuromorphology in hippocampal neurons, suggesting that these drugs can be used interchangeable.

Ref#1: The authors indicate that miR129-5p has been previously identified as a regulator of potassium channel Kv1.1 expression (Sosanya et al. 2013) but they do not show their own results. Looking at their RNA-Seq data, it shows an up-regulation of Kv1.1 after PTX treatment. This result is opposite to the expected regulation in this condition where miR-129-5p is up-regulated. Authors should comment on that.
We agree that Kv1.1 mRNA upregulation by PTX is contrary to expectation, since elevated miR-129-5p levels should lead to Kv1.1. repression. One possible explanation for this discrepancy is that miR-129-dependent repression of Kv1.1 does not involve mRNA degradation, but rather occurs solely at the level of mRNA translation. This could also be related to the dendritic localization of Kv1.1, since mRNA levels of other dendritic miRNA targets (e.g. Limk1) are similarly not affected by miRNAs (Schratt et al., 2006). In support of this hypothesis, Sosanya et al. did not find any changes in Kv1.1. mRNA stability in response to rapamycin treatment or mutation of the miR-129 seed match within the Kv1.1 3'UTR. Sustained suppression of mRNA translation under certain conditions, e.g. stress, can even paradoxically lead to mRNA stabilization (reviewed in Huch and Nissan, Wiley Interdiscip Rev RNA, 2014), which could explain the observed increased Kv1.1 levels in our study. We have now added this in the discussion of our revised manuscript (p.18-19).

Ref#1: Authors investigated target mRNAs of miR-129-5p with a nice set of experiments. Their demonstration that miR-129-5p is targeting Atp2b4 is really convincing. In contrast, Dcx targeting is less obvious. In particular, the luciferase experiment shows a regulation of Dcx-reporter even when miR-129-5p site is mutated (Fig. 5D) and the associated statistic is, in my opinion, not well performed. Indeed, authors have to compare the results obtained with WT reporter and mutated reporter, but they did two separate one-sample t-tests for WT and mutated reporters. Since this kind of test is less stringent, authors should confirm that result by directly comparing the two conditions with a t-test (or a non-parametric test if distribution is not normal).

Our response: Following the suggestion of this reviewer, we have now compared also wild-type and mutant reporters by t-test. This analysis revealed a significant difference for Atp2b4, but not Dcx (Fig. 5D). Similar effects were observed in PTX-treated neurons (Fig. 5E). Our interpretation of this data is that in contrast to Atp2b4, downregulation of Dcx during synaptic downscaling is not absolutely dependent on miR-129-5p. However, we observe a significant downregulation of Dcx protein and 3'UTR reporters by miR-129-5p overexpression (Fig. 5C, D), which could be primarily due to an inactivation of Rbfox proteins, which stabilize the Dcx mRNA. The strong stabilization effect of Rbfox proteins on Dcx is further demonstrated by our Rbfox1 knockdown experiments (Fig. 7D, E). Therefore, our experiments are consistent with a negative effect of miR-129-5p on Dcx, but this effect is likely mediated primarily by downregulation of Rbfox proteins, and only to a minor extent by a direct action of miR-129-5p on the Dcx 3'UTR. We have now included this also in the revised manuscript (p.13; p.18).

Ref#1: To summarize their findings, authors did a scheme in Fig. 8G that suggests a regulation of Pp3ca, Rbmx and Celf1 by miR-129, miR-212 and miR-543. However, their data do not support this regulation pathway. A full demonstration, using at luciferase reporter and/or over-expression of candidate miRNAs, should be performed before drawing this conclusion.
Our response: We fully agree that we do not have any experimental support at this point for a regulatory function of additional PTX-regulated miRNAs, like miR-212 and miR-543, on targets like Pp3ca, Rbmx and Celf1. An in-depth study of these miRNAs would require extensive additional experimentation, which is clearly beyond the scope of the present study. We nevertheless included these predicted interactions into our model in order to illustrate that additional pathways, other than Rbfox/miR-129-5p, are likely involved in PTX-dependent post-transcriptional repression during synaptic scaling. However, to tone down our conclusions, we have now added questions marks at pathways without experimental support and moved the entire model into the supplementary part (suppl. Fig. S6F).

Minor points:
Ref#1: In the introduction: homeostasis is not only involved in development and injuries but also in learning mechanisms in physiological conditions.
Our response: We fully agree and have now added a very recent reference that shows the importance of synaptic scaling-down for memory consolidation during sleep (Diering et al., Science 2017) in the introduction (p. 4) of the revised manuscript.

Ref#1: It is not clear how authors did miRNA screening in PTX model, is this based on classical RNA extraction or on Ago2-IP? Please, clarify.
Our response: We apologize for not being clear here. Small RNA sequencing for miRNA profiling was performed with total RNA obtained with classical extraction, Ago2-IP was only performed in the rat PPS epilepsy model (Fig. 3A). We have now clarified this in the text and methods section.

Ref#1: It is not clear how authors selected PTX-downregulated genes from RNA-Seq data to perform qRT-PCR.
Our response: We first intersected RNAseq and proteomics datasets to narrow down on important genes. Among the commonly regulated genes, we focused on ones that were already implicated in processes related to synaptic scaling, e.g. calcium homeostasis (Atp2b4) and signaling (Camk2a/b), as well as cytoskeletal remodeling (Dcx).

Ref#1: An interesting finding is the anti-correlation of miR-129-5p and Atp2b4 in human TLE patients. Since authors try to demonstrate that Dcx is also a target of miR-129-5p, it would be interesting to quantify Dcx levels as well.
Our response: We have now performed also Dcx qPCR in human TLE patients and found that Dcx and miR-129-5p levels were significantly anti-correlated (Pearson's r=-0.745; p=0.034), (Fig. 5H). This suggests that Dcx regulation by miR-129-5p (presumably via Rbfox downregulation) could also be relevant in the context of human epilepsy.

Referee #2:

Ref#2: 1. The discussion is very high level and does not comment or expand on the data collected.
Our response: We have now extensively re-written the discussion, taking into consideration points raised by the two reviewers and further paying more attention to subjects that directly related to our data.

Ref#2: 2. Two examples are presented in figure 2. The authors claim that miR-129-5p over expression suppresses PTX dependent down scaling of dendritic spine as well as mEPSC amplitude, yet it is clear from the images and such that miR-129-5p over-expression alone does this, making it difficult to conclude that miR-129-5p has specifically block PTX dependent down scaling or something more general. As such the claim is not supported by the data.
Our response: The observation of the reviewer that miR-129-5p inhibition already reduces spine size under basal conditions is correct. We have now also included a different figure of spine size quantification, in which we present separate bars for vehicle- and PTX-treated neurons, to better illustrate this fact (suppl. Fig. S1B). Therefore, in addition to its requirement in PTX and Bic-dependent downscaling of spines, miR-129-5p likely possesses an additional positive function during spine development. In addition, we now also performed miR-129-5p overexpression by mimic transfection, and found that miR-129-5p was sufficient to induce spine shrinkage in the absence of PTX (suppl. Fig. S1C). Taken together, while multiple lines of evidence argue for an important function of miR-129-5p in the homeostatic downscaling of dendritic spines, additional developmental functions of miR-129-5p might be superimposed. We are acknowledging this on p.7 of our revised manuscript.

Ref#2: 3. It is unclear why the authors change their seizure inducing models. They make a big deal about using electrical stimulation but then switch to a kainic acid model for all other assay. Some explanation is required. It is also unclear why the authors think that PBS injection is a proper control for their Anti-miR-129 injections. Seems that a scrambled or mutant oligo would be more appropriate.
Our response: We have initially decided for the PPS model, since it is probably the model that most closely resembles human temporal lobe epilepsy. However, for functional experiments, we focused on the kainic acid (KA) model, since this is experimentally less challenging, time-consuming and resource intensive. Importantly, in contrast to PPS, the KA model has already been successfully used in the context of anti-miR injection by our long-standing collaborator D. Henshall (RCSI Dublin) in multiple studies (Jimenez-Mateos et al., 2011, 2012, 2015; McKiernan et al., 2012), providing us with a framework for conceiving the experiments. To complement our analysis, we have now also included qPCR data that show significant increases in Ago2-associated miR-129-5p upon intra-amygdalar KA injection (suppl. Fig. S2C).
Concerning the control for anti-miR-129 injections, we are confident that PBS is an appropriate control, since we did not observe any statistically significant differences between PBS and anti-miR control injections on various EEG parameters in a different animal cohort (suppl. Fig. S3).

Ref#2: 4. The images in figure 6 have the same problem as the authors do not address the over-expression effects of Atp2b4 and Dcx on spine number and mEPSC amplitudes in control treated cells. Also the data in 6A and 6B are duplicated and should be merged into one panel.
Our response: Similar to Fig.2A, we now provide a different figure for spine size quantification (suppl. Fig. S6E) in which we present separate bars for vehicle- and PTX-treated neurons. Statistical analysis revealed no significant differences in spine size between overexpression of Atp2b4 or Dcx and control-transfected cells in the absence of PTX (suppl. Fig. S6E). This suggests that downregulation of these proteins is selectively required for spine shrinkage in PTX-treated neurons. We further apologize for the duplication mistake in Fig. 6A/B and have now merged the respective data (which originated from the same neuron transfections) into one figure panel (Fig. 6A, upper panel).

Ref#2: 5. On page 19 in the discussion the authors bring up a new topic not found in the results, namely that there is a global reduction of the neuronal calcium extrusion system. If this is such an important point to make, the some reference in the results should be made.

Our response: We have now made a reference on p.19 to Fig. 4B and suppl. Fig. S5J (GO-term enrichment analysis) in the results part, where "regulation of calcium-mediated signaling" showed up as one of the most significant pathways in the set of PTX-downregulated mRNAs respective proteins.

2nd Editorial Decision 31 March 2017

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been re-reviewed by the two referees and their comments are provided below.

As you can see the referees appreciate the introduced changes. I am therefore very happy to let you know that we will accept the manuscript for publication here.

Congratulations on a a nice study

REFEREE REPORTS

Referee #1:

In this revised version of their manuscript, Rajman et al. addressed all my concerns about their interesting results and is now, in my opinion, suitable for publication.

Referee #2:

This is an excellent study designed to assess the contribution of miRNAs in the translational control of gene/mRNAs involved in homeostatic down scaling of genes associated with elevated network activity, e.g. during epilepsy. The data presented are of high quality, with a solid set of controls. The authors have nicely address the previous reviewer concerns. I recommend publication without further revision.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name:	Gerhard Schratt
Journal Submitted to:	EMBO Journal
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Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ 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.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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?	Samples sizes are appropriate for the relevant data sets, as can be determined by comparison with multiple related peer reviewed studies. In most of the cases 3 repetitions were used. If variability was higher 4-6 replicates were used. In Fig. 3H experiment - comparing human samples. We obtained tissue from 10 control patients and 6 patients with epilepsy + HS. In Fig. 5G analysis - used data from 15 epilepsy patients; In Fig. 5H analysis - used data from 8 epilpesy patients
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Group sizes are appropriate for the relevant data sets, as can be determined by comparison with multiple related peer reviewed studies
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	not applicable
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	not applicable
For animal studies, include a statement about randomization even if no randomization was used.	In the animal studies, the animals were assigned to the various groups randomly. In imaging studies, cell selection was performed randomly, excluding unhealthy cells and cells not easily identified as pyramidal neurons. Data was collected and processed randomly (Appendix methods).
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.	for all microscopy and patch-clamp analysis, investigator was blinded to the experimental conditions (stated in Appendix methods)
4.b. For animal studies, include a statement about blinding even if no blinding was done	For all mice studies investigator was blinded to the treatment.
5. For every figure, are statistical tests justified as appropriate?	yes, see methods or respective figure legends
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	For most of the experiments data distribution was assumed to be normal. When experiment contained more data points we tested it for normality. (Appendix, "Statistics").
Is there an estimate of variation within each group of data?	reported below

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Is the variance similar between the groups that are being statistically compared?	In the case of one-sample t-test we were comparing change vs 1 (not change). In this case we always assumed heteroscedasticity. In the case of two-sample t-test, beacuse of low number of biological replicates (3-5; just in one case Fig 6J n=6,10) we could not reliably test for differences in variance between groups and therefore we assumed homoscedascity. In the case of GLM analysis and one-way ANOVA we assumed homoscedascity.

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).	yes, only validated antibodies from commercial sources were used in this study.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	not applicable

* 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.	Wistar, Sprague Dawley, purchased from Harlan and Charles River, respectively) reported in the online methods section ("Animal experiments") C57BL/6JJ mice - purchased from Harlan, UK
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Yes, methods section, first paragraph
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 that we consulted ARRIVE guidelines and ensure that all animal studies were accordingly performed, as stated in methods section.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Human studies were approved by the Ethics (Medical Research) committee of Beaumont hospital (REC#13-75).
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.	Written, informed consent was obtained from all subjects.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Inclusion Criteria. 1. Patients must be attending the epilepsy service at Beaumont Hospital.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	not applicable
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.	not applicable
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.	not applicable

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	all data from proteomics and genomics experiments were deposited to relevant databases (Data access section)
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).	not applicable
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).	not applicable
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	methods "CLIP analysis"
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Yes (Appendix Methods: "CLIP") To analyze results from: 3'UTR length estimation (Appendix methods) miRNA seeds (Appendix methods) GO-Terms (Appendix methods) Custom scripts are provided in a repository available on github https://github.com/dieterich-lab/rajman_et_al

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	not applicable