THE EMBO JOURNAL

Jacob-induced transcriptional inactivation of CREB promotes Aβ-induced synapse loss in Alzheimer's Disease

Katarzyna Grochowska, Guilherme Monteiro Gomes, Rajeev Raman, Rahul Kaushik, Liudmila Sosulina, Hiroshi Kaneko, Anja Oelschlegel, PingAn Yuanxiang, Irene Reyes Resina, Gonca Bayraktar, Sebastian Samer, Christina Spilker, Marcel Woo, Markus Morawski, Jürgen Goldschmidt, Manuel Friese, Steffen Roßner, Gemma Navarro, Stefan Remy, Carsten Reissner, Anna Karpova, and Michael Kreutz
DOI: 10.15252/embj.2022112453

Corresponding author(s): Michael Kreutz (kreutz@lin-magdeburg.de) , Anna Karpova (anna.karpova@lin-magdeburg.de)

Review Timeline:

Submission Date:	25th Aug 22
Editorial Decision:	15th Sep 22
Revision Received:	13th Nov 22
Editorial Decision:	30th Nov 22
Revision Received:	7th Dec 22
Accepted:	9th Dec 22

Editor: Karin Dumstrei

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. 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 **15th Sep 2022**

Dear Michael,

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

As you can see from the comments, the referees appreciate the findings and are supportive of publication here. Referee #1 raises some important concerns regarding the in vivo work that I would like to ask you to address in a revised version.

I think it would be helpful to discuss the revisions further and I am available to do so via email or a video call. Let me know what works best for you.

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: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

I thank you for the opportunity to consider your work for publication. I look forward to discussing your revisions further with you.

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Our source data coordinator will get in touch with you to discuss what figures would be good to have source data for.

I have also attached a guide with helpful tips on how to prepare the revised version.

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (14th Dec 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

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.

Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

In this manuscript, the authors show the involvement of Jacob in Alzheimer's Disease synaptic failure. They describe a molecular mechanism implying Aβ-induced extrasynaptic NMDAR activation and nuclear import of Jacob for the induction of CREB shutoff. They found that Jacob interacts with LMO4, a transcriptional co-activator of CREB. LMO4 hinders dephosphorylation of S133, stabilize the CREB dimer and thereby could act as transcriptional enhancer. In the presence of amyloid pathology Jacob likely displaces LMO4 from the CREB complex. Taking advantage of structural modelling, they selected Nitarsone, a small chemical compound that selectively interrupts the interaction of Jacob, but not of CREB with the LIM1 domain of LMO4. They provide also data showing the therapeutic potential of Nitarsone administration to prevent Aβ-induced synaptic failure and cognitive deficit in AD mouse models.
The article is interesting and paves the way to novel therapeutic targets for AD treatment.
However, in the present form the manuscript is extremely complicated, difficult to read and therefore the overall message is somehow lost.

General issues:
Statistical issue: when the authors show statistical analysis of experiments performed in animals, it appears that they consider data obtained from the individual sections as experimental units instead of replicates, which is a statistical pseudoreplication of the data. Are the results still the same when the animal is used as the experimental unit or when a statistical model is used that takes into account the animal (for example linear mixed effects models?).
Specific issues:
Fig. 1 A-E
Why the authors analyzed Jacob and CREB pathway in the cortex of AD patients when they focus on the role of this mechanism in the hippocampal region?
Which is the Braak stage of the patients? And which cortical area did they analyze?
Which are the levels of p-Jacob and Jacob in NeuN positive nuclei? In this form the experiments presented in Fig 1 are not useful for the main message.
Fig. 1G-H How long the authors treated the cells with Amyloid-beta oligomers? Why did the authors used a different concentration of Amyloid beta oligomers to treat organotypic slices (1uM, Fig. 1I)?
Fig. EV1 M-N The analysis of cerebral flow as functional outcome is not acceptable as functional rescue outcome. It is mandatory to analyse the effect of Jacob deficiency on cognitive function performing behavioral tests reported in Fig.8 (Nitarsone treatment).
Fig.2-4 Coimmunoprecipitation experiments showing the association of Jacob and CREB, Jacob and LMO4 and Jacob and PP1 were performed in heterologous cells overexpressing the proteins. Despite the elegance of all in vitro experiments which indeed very convincing, it is mandatory to demonstrate that these proteins are also interacting in physiological conditions, in brain tissue or in neuronal cells by coimmunoprecipitation assays or proximity-ligation assays
Fig. 2 O-R. Does the LMO4 binding mutant of Jacob localize in the nucleus in the same extent of the non-mutate Jacob?
Fig.3-4 The authors show that the N-terminus of Jacob (fig.3) and the phosphomimetic Jacob (Fig. 4) displace LMO4 from CREB performing an in vitro assay, but it is critical to demonstrate this competition in live cells. As indicated in comments of Fig 2-4 in vivo experiments are essential to convince this referee of the effect of Nitarsone for AD
Fig.5-6 The efficacy of Nitarsone in interfering with LMO4/Jacob binding without affecting the association to CREB was demonstrated taking advantage of in vitro assays. Target engagement should be demonstrated in neuronal cultures and in mice receiving Nitarsone by coimmunoprecipitation assays.
Fig. 7A Why did the authors chose a different administration protocol for TBA2.1 and 5xFAD mice? Why did the mice single caged before performing behavioural tests? Did the authors take into consideration the stress induced by the single caging? The administration protocol reported in materials and methods is not clear.

In general, the manuscript contains elegant in vitro demonstration of potential role of Nitarsone, nonetheless the final proof of in vivo effects as well as target confirmation in both animal models and patients is still lacking, diminishing the enthusiasm for the results shown.

Referee #2:

This is a careful and thorough study delineating a specific pathway via which the cytonuclear factor Jacob can regulate the transcription factor CREB. Experiments are well laid out and there is significant and sufficient experimental evidence to support the claims. Furthermore, the team uses structural modeling and bioinformatic analyses to identify a compound that specifically interferes with the protein complex described and uses it to test the hypothesis that Jacob mediates the deleterious actions of Amyloid beta peptides on neuronal function.

Overall, the study is excellent. There is a wealth of mechanistic insights presented that detail a set of interactions between Jacob, LMO4, and CREB, and describe how this complex may be important in Alzheimer's pathophysiology using two different AD animal models. This reviewer has only relatively minor points that should be addressed:

MINOR:
1- References included (Saura 2011, Caccamo 2010, Bartolotti 2016) and not included (Pugazhenti 2011) show that total CREB levels are downregulated in AD pathology in both humans and mice. These changes may explain reduced pCREB levels. The authors should mention that their results differ from this (Fig 1E) and provide some explanation for the differences.

2- Because truncations may impact protein stability, FRET experiments in Fig2 and Fig3 should be normalized to the amount of construct that is expressed.

3- Line plots of STED images (EV2D,EV3D,E) are not very informative. Since conclusions are drawn from these data, quantitative measures of colocalization should be included.

4- The amount of Jacob expressed in Fig 2(N-R) needs to be considered for results.

5- The intent and interpretation of the Nitarsone pretreatment vs posttreatment experiments is not clear. If pretreatment works, does that mean that the Nitarsone effects are irreversible, or does the drug not wash out? Could the sustained effects on the Jacob/LMO4/CREB complex be potentially deleterious over the long run? This section needs additional interpretation.

6- In behavioral assays, statistical analyses comparing before and after treatment with nitarsone (Fig 8H-P) are needed (not just comparing across genotypes). Two-way ANOVAs should be used.

7- Overall, statistical analyses should be better described, specifically the types of tests performed for each experiment.

8- The effects of Nitarsone on excitability may be difficult to interpret. Metabolic byproducts of Nitarsone include ionic Arsenic, which can block certain K+ channels (and potentially other channels). This caveat should be mentioned in the discussion.
9- In this reviewer's opinion, the paragraphs on Nitarsone in the discussion weaken the paper and should be removed. Proposing Nitarsone as a therapeutic option based on one set of experiments is not needed for the strength of this work and considerably overstates the issue (which may even be dangerous).

10-Small things- Fig 1E: There is no statistical analyses. Fig 1C: Normalized levels for pJacob/Jacob should have a value of 1 (If not, why not?). FigEV6: legends are switched. Fig 8A: Legend is not complete.

Referee #3:

The authors report that soluble Aβ elicits cytonuclear trafficking of Jacob, which by acting as a mobile signaling hub docks a signalosome to CREB, producing transcriptional inactivation and subsequent synapse impairment and eventually loss in AD.

They also report that Nitarsone, by selectively hindering the assembly of this signalosome, restores CREB transcriptional activity. In addition, they report that Nitarsone prevents the impairment of synaptic plasticity and the cognitive decline displayed by AD mouse models.

They suggest that targeting the CREB shutoff induced by Jacob represents a therapeutic avenue against the early synaptic dysfunction in AD.

This is excellent work. I have no major suggestions to make. Hence, I recommend publication in its present form.

Only a few writing errors should be corrected, such as lack of spaces between words and between words and references.

Referee #1:

In this manuscript, the authors show the involvement of Jacob in Alzheimer's Disease synaptic failure. They describe a molecular mechanism implying Aβ-induced extrasynaptic NMDAR activation and nuclear import of Jacob for the induction of CREB shutoff. They found that Jacob interacts with LMO4, a transcriptional co-activator of CREB. LMO4 hinders dephosphorylation of S133, stabilize the CREB dimer and thereby could act as transcriptional enhancer. In the presence of amyloid pathology Jacob likely displaces LMO4 from the CREB complex. Taking advantage of structural modelling, they selected Nitarsone, a small chemical compound that selectively interrupts the interaction of Jacob, but not of CREB with the LIM1 domain of LMO4. They provide also data showing the therapeutic potential of Nitarsone administration to prevent Aβ-induced synaptic failure and cognitive deficit in AD mouse models. The article is interesting and paves the way to novel therapeutic targets for AD treatment. However, in the present form the manuscript is extremely complicated, difficult to read and therefore the overall message is somehow lost.

Reply: We want to thank the Referee for the positive comments. We have tried to further improve the flow of arguments. In the first draft we have introduced to this end summary cartoons in most Figures to illustrate the experimental outline. Moreover, we summarize either at the end or beginning of each sub-section in Results the key findings.

General issues:
Statistical issue: when the authors show statistical analysis of experiments performed in animals, it appears that they consider data obtained from the individual sections as experimental units instead of replicates, which is a statistical pseudoreplication of the data. Are the results still the same when the animal is used as the experimental unit or when a statistical model is used that takes into account the animal (for example linear mixed effects models?).

Reply: We thank the Referee for pointing this out. Following slicing all brain sections were collected in 24 well-plate (single slice per well) and proceeded for staining separately. Therefore, we considered individual sections as one experimental unit. From each mouse on average 2 sections per readout were used which were approximately 105 µm apart from each other. Thus, averaging of the data has also some downsides. Moreover, although at first glance, the differences look rather modest, we were surprised to find these differences at all at this very early stage of AD progression, given that only a limited number of cells are affected at this stage. Increasing N-number of animals will be very difficult because of time constrains (we need at least 20 animals per group) and 3R principles. Nevertheless, we have addressed this issue and re-analyzed the data from the figures where more than one data point derived from a single animal (Fig. 1L-P, Fig. 7C-L, Fig EV1O-S, and Fig EV7C-N) using linear mixed-effects model (LMM) as suggested by the referee. In addition, when possible, we have analyzed additional slices from some animals to ensure comparable number of slices from all animals. Therefore Fig. 1M, O, P and Fig. 7H, J were revised in the manuscript. In all cases the effects remained significant, in some cases however, due to the effect size, the p-value increased. Taking into consideration the early stage of AD, where not all neurons are yet affected, we still consider the differences stunning and would further stress the major contribution of the described pathway to the onset of AD pathology. We have updated the statistics in the graphs and in the figure legends of the manuscript.

Specific issues:
Fig. 1 A-E
Why the authors analyzed Jacob and CREB pathway in the cortex of AD patients when they focus on the role of this mechanism in the hippocampal region?

Reply: The purpose of this experiment was to show that the phenomena under study indeed exists in human brain. Of note, CREB shutoff has not been shown previously in specimens from human AD patient brain. The samples from human brains come from patients with advanced AD pathology and hippocampal tissue as such and from patients at a very early stage of the disease is not available. Additionally, the temporal cortex is one of the brain regions prominently affected in AD and in practical terms provides sufficient amounts of brain tissue necessary for the sample preparation.

Which is the Braak stage of the patients?

Reply: We have added this information to the corresponding Table S1 in the revised version of the manuscript.

And which cortical area did they analyze?

Reply: We have analyzed the temporal cortex area 22. The information was previously available in the Table S2, section "biological samples". We have now included it additionally in the title of the Table S1.

Which are the levels of p-Jacob and Jacob in NeuN positive nuclei? In this form the experiments presented in Fig 1 are not useful for the main message.

Reply: We think this is a misunderstanding. Our previous work has shown that Jacob is exclusively expressed in neurons and not in glia (Mikhaylova et al, 2014). Moreover, previous analysis suggests that pJacob is most prominent in neuronal nuclei as a consequence of synapto-nuclear shuttling (Karpova et al., 2013). In addition, we do not have FACS data because there is currently no antibody available that is suitable for this application but given the expression pattern of pJacob we also don't see an ultimate need to perform such experiments.

Fig. 1G-H How long the authors treated the cells with Amyloid-beta oligomers? Why did the authors used a different concentration of Amyloid beta oligomers to treat organotypic slices (1uM, Fig. 1I)?

Reply: Organotypic hippocampal slice cultures (OHSCs) were treated with Amyloid-β (Aβ) oligomers for 1h, as indicated in the figure legend and methods section. We have added this information to the main text of the revised version of the manuscript. The differences in concentration of Aβ oligomers are due to the different culture systems. In dissociated cultures, neurons form a single layer, and Aβ is applied directly to the cell culture media and rapidly accumulates on neuronal membranes. In OHSCs, the tissue slices are thick (300-400 µm) and grow on a semi-permeable membrane, so they are not in direct contact with the media. Therefore, higher concentrations of Aβ were chosen to ensure that sufficient amounts of the oligomers will penetrate the glia layer surrounding the tissue and reach neuronal membranes in the center of the slice.

Fig. EV1 M-N The analysis of cerebral flow as functional outcome is not acceptable as functional rescue outcome. It is mandatory to analyse the effect of Jacob deficiency on cognitive function performing behavioral tests reported in Fig.8 (Nitarsone treatment).

Reply: We want to respectfully disagree with this statement of the Referee. On our opinion it is a functional rescue - just different from the function that the reviewer is requesting. We generated double transgenic line (Jacob/Nsmf knock out crossed with TBA2.1 line) to test the relevance of the Jacob pathway for CREB shutoff in mouse brains. However, one has to note that Jacob gene knockout itself results in impairments of LTP and cognitive function (Spilker et al., 2016), so the results would be hard to interpret. This impairment can be easily explained because the mice lack synapto-nuclear transport of pJacob from synaptic NMDAR that is induced by LTP, which in turn results in increased CREB-dependent plasticity-relevant gene expression (Behnisch et al., 2011, Karpova et al., 2013, Spilker et al., 2016).

Fig.2-4 Coimmunoprecipitation experiments showing the association of Jacob and CREB, Jacob and LMO4 and Jacob and PP1 were performed in heterologous cells overexpressing the proteins. Despite the elegance of all in vitro experiments which indeed very convincing, it is mandatory to demonstrate that these proteins are also interacting in physiological conditions, in brain tissue or in neuronal cells by coimmunoprecipitation assays or proximity-ligation assays

Reply: We thank the Referee for the positive comment. As suggested by the Referee we have used proximity ligation assays to demonstrate a tight association between proteins in a specific subcellular compartment. We employed primary hippocampal cell culture and PLA to confirm the interaction between Jacob and CREB under basal conditions. To this end we have used rabbit anti-Jacob antibodies that were successfully tested previously for a similar application (Samer et al., 2021). We have updated Figure 2 and added panel G showing the direct interaction of Jacob with CREB in neuronal nuclei. In addition, we performed PLA for the detection of a direct interaction between Jacob (anti-Jacob, rb) and LMO4 (anti-LMO4, goat) and added a new "L" panel to Figure 2. Unfortunately, PLA has a particular limitation based on primary antibody suitability for this assay. Thus, the anti-PP1γ antibody is not suitable for this assay due to its host species. Moreover, the anti-Jacob antibody is not suitable to perform PLA on brain sections from transgenic mice. However, we could show PLA signals for CREB-LMO4 (two examples included for the Referee see below, scale bar 10 µm), i) confirming the previously published functional interaction (Kashani et al., 2006), ii) our in vitro data showing a direct interaction and iii) supporting the validity of the PLA assay concerning the other interactions.

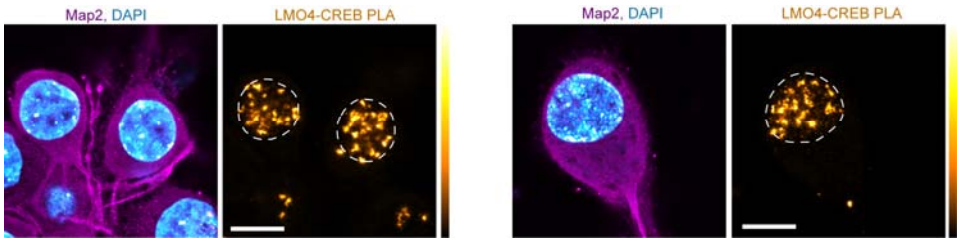


Fig. 2 O-R. Does the LMO4 binding mutant of Jacob localize in the nucleus in the same extent of the non-mutate Jacob?

Reply: Yes. This can be also seen in the GFP-channel. The LMO4 binding region is not overlapping with the nuclear localization signal that is critical for nuclear translocation of Jacob (Dieterich et al., 2008). Therefore, this mutation will not to alter the nuclear localization of Jacob. We have included below an overview of all images for the GFP and DAPI (nuclear marker) channel for the Referee. The lookup table indicates the pixel intensities from 0 to 255, scale bar 10 µm.

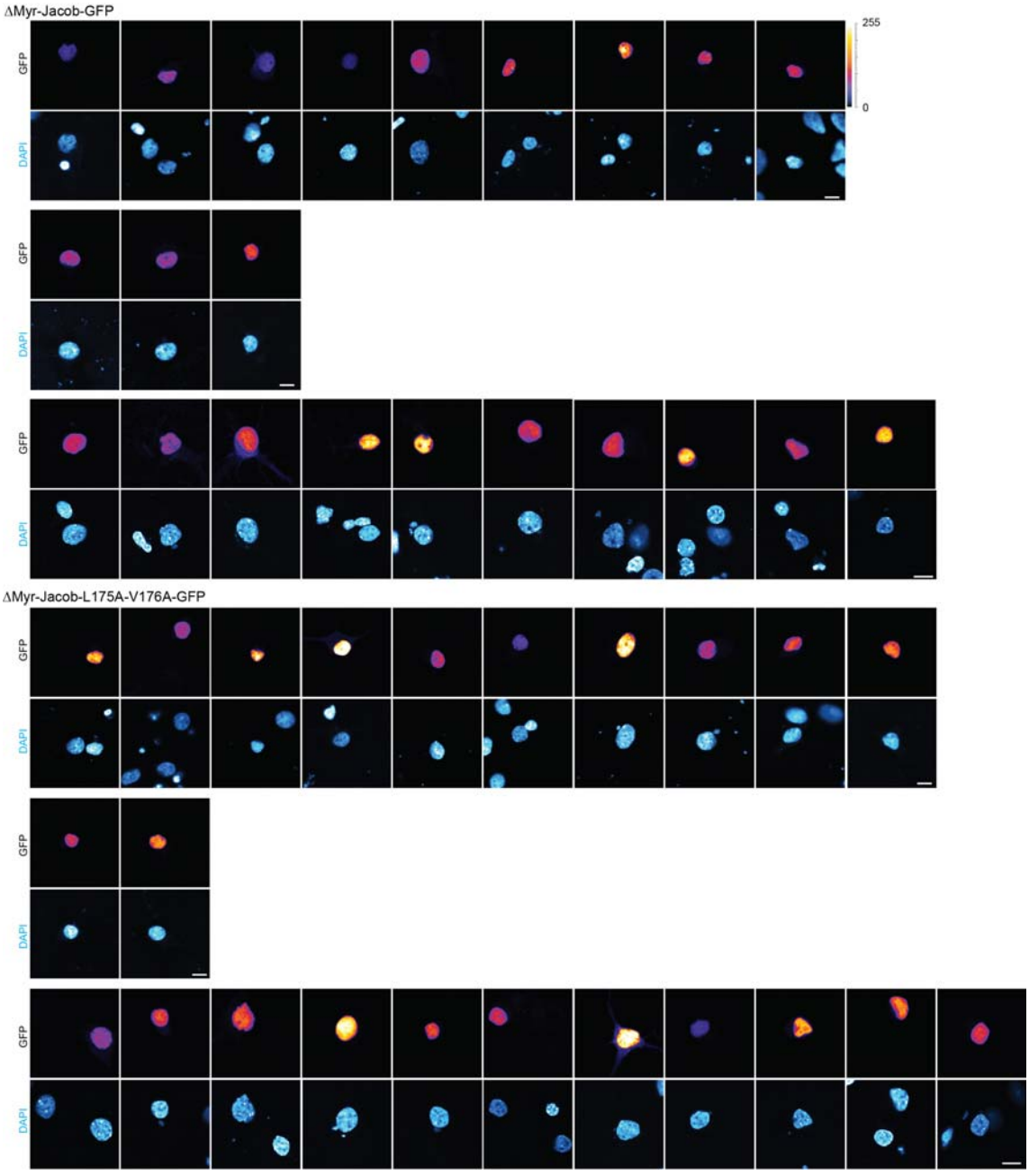


Fig.3-4 The authors show that the N-terminus of Jacob (fig.3) and the phosphomimetic Jacob (Fig. 4) displace LMO4 from CREB performing an in vitro assay, but it is critical to demonstrate this competition in live cells. As indicated in comments of Fig 2-4 in vivo experiments are essential to convince this referee of the effect of Nitarsone for AD

Reply: We think that there is a misunderstanding. In Fig. 4, we show that phosphodeficient, and not phosphomimetic Jacob binds more efficiently to LMO4 (panel 4F, G) and displace LMO4 from CREB (Fig 3N-Q). In contrast, the phosphomimetic Jacob mutant does not displace LMO4 from CREB (panel H-J). We show the displacement in living cells (SRET – Fig. 3L-N). Nonetheless we addressed the issue raised by the reveviewer by employing Jacob-LMO4 PLA in primary neurons treated with Aβ (500 nM) for 48 h plus/minus Nitarsone (5 μM). Aβ treatment enhances dephosphorylation of Jacob (Grochowska et al., 2017) and this treatment results in a dramatic increase in PLA puncta indicating increased association. Intriguingly, this was not the case in the presence of Nitarsone supporting the idea that Nitarsone block the binding pocket in LMO4 for association with Jacob. We have added panel N and O to Figure 5 and updated the Figure legend.

Fig.5-6 The efficacy of Nitarsone in interfering with LMO4/Jacob binding without affecting the association to CREB was demonstrated taking advantage of in vitro assays. Target engagement should be demonstrated in neuronal cultures and in mice receiving Nitarsone by coimmunoprecipitation assays.

Reply: We appreciate the Referee's comment, although we would like to express several concerns. First, endogenous co-immunoprecipitation is not a very quantitative method. We cannot exclude that we following the rather harsh extraction from neuronal nuclei will wash out Nitarsone from the complex. Consequently, Jacob might easily bind again to the LIM1 domain of LMO4. Second, for performing experiments with animals, we would need an amendment of the license allowing the treatment of a new batch of mice with Nitarsone. Thus, this experiment will be difficult to perform due to time constraints and it will take more than three months. However, as outlined above we have performed PLA assays to show the effective displacement of Jacob from LMO4 following Nitarsone treatment in neuronal primary neurons (updated Figure 5 N, O).

Fig. 7A Why did the authors chose a different administration protocol for TBA2.1 and 5xFAD mice? Why did the mice single caged before performing behavioural tests? Did the authors take into consideration the stress induced by the single caging?

Reply: The treatment of TBA2.1 mice started with 4 weeks of age whereas treatment of 5xFAD mice started with 12 weeks old mice. We had to adapt the administration protocols based on the available literature due to the difference in the onset of pathology between both transgenic lines. The Nitarsone administration was either by forced feeding or voluntary. Voluntary feeding was preferred due to animal welfare considerations and for reasons of convenience. Animals that were group housed had to be force fed to ensure equal administration of the drug. Females and young animals suffer more from single housing than adult males (who are barely if at all afflicted). When we employed TBA2.1 animals we used both sexes and had to start at a young age. The mice were therefore group housed first and forced fed. The 5xFAD mice display sex-specific differences in pathogenesis. Therefore, we focused on male mice which could be single housed from the start and thus voluntary feeding was an option.
In order to provide equal conditions and to avoid the influence of stress caused by fights for group status, all (TBA2.1 & 5xFAD) mice were single housed prior to behavior experiments. In order to allow some time for adjustment to the new housing condition and thereby reduce stress, we started single housing a week prior to the behavior experiments. Although single

housing may induce stress in the female mice, we did not observe any gender-specific behavioral differences within the TBA2.1 mice group.

The administration protocol reported in materials and methods is not clear.

Reply: We apologize that the administration protocol was not clear from the beginning. We provide more detailed information in the revised version of the manuscript.

In general, the manuscript contains elegant in vitro demonstration of potential role of Nitarsone, nonetheless the final proof of in vivo effects as well as target confirmation in both animal models and patients is still lacking, diminishing the enthusiasm for the results shown.

Reply: We hope that we could convince the Referee that the Nitrasone administration disrupts the interaction between non-phosphorylated Jacob and LMO4 as revealed by proximity ligation assays in neurons and, therefore, will prevent displacement of LMO4 from CREB keeping CREB transcriptionally active.

Referee #2:

This is a careful and thorough study delineating a specific pathway via which the cytonuclear factor Jacob can regulate the transcription factor CREB. Experiments are well laid out and there is significant and sufficient experimental evidence to support the claims. Furthermore, the team uses structural modeling and bioinformatic analyses to identify a compound that specifically interferes with the protein complex described and uses it to test the hypothesis that Jacob mediates the deleterious actions of Amyloid beta peptides on neuronal function.

Overall, the study is excellent. There is a wealth of mechanistic insights presented that detail a set of interactions between Jacob, LMO4, and CREB, and describe how this complex may be important in Alzheimer's pathophysiology using two different AD animal models. This reviewer has only relatively minor points that should be addressed:

Reply: We would like to thank the Referee for the very positive comments.

MINOR:
1- References included (Saura 2011, Caccamo 2010, Bartolotti 2016) and not included (Pugazhenti 2011) show that total CREB levels are downregulated in AD pathology in both humans and mice. These changes may explain reduced pCREB levels. The authors should mention that their results differ from this (Fig 1E) and provide some explanation for the differences.

Reply: We are a bit confused by this statement. We carefully went through the figures of the cited papers and we don't think that the literature suggests lower CREB levels in human AD and in mouse models of AD.

1) *Saura 2011 – review –discusses mainly CREB activation and its Ser133 phosphorylation in various mice models and in vitro studies of AD. There is only one sentence where the downregulation is mentioned: "There is compelling evidence indicating reduced levels of total or phosphorylated CREB in the hippocampus of old mice and rats (Brightwell et al. , 2004 ; Kudo et al. , 2005 ; Porte et al. , 2008) (…)".*

Brightwell et al., Kudo et al. and Porte et al. do not analyze transgenic AD mice but investigate CREB in senescent animals.

2) *Caccamo 2010 – the authors focus on mouse model of AD - 3xTg-AD mice. In the panels (Fig. 1B,C; Fig. 4A) the decrease in pCREB (Ser133) but not downregulation of CREB is reported.*
3) *Bartolotti et al., 2016 – is a study on mice (APPswe/PS1d9 mice). The following panels (Fig. 1A, B; 2C, D) display the change in pCREB but not total CREB.*
4) *Pugazhenti et al., 2011 – report in Fig. 1 – decreased CREB mRNA in a AD mouse model (Tg2576). The other figures show either total homogenate, including CREB from glial cells (Fig. 2A, 4A), or show different brain regions (DG – Fig. 2C). In addition the in vitro model is hard to compare as they use 2 μM fibrils whereas in our study 500 nM oligomers were used.*

Taken together we don't see a discrepancy between the literature and our data in particular if one takes into account that we have worked with mice at a very early stage of AD pathology.

2- Because truncations may impact protein stability, FRET experiments in Fig2 and Fig3 should be normalized to the amount of construct that is expressed.

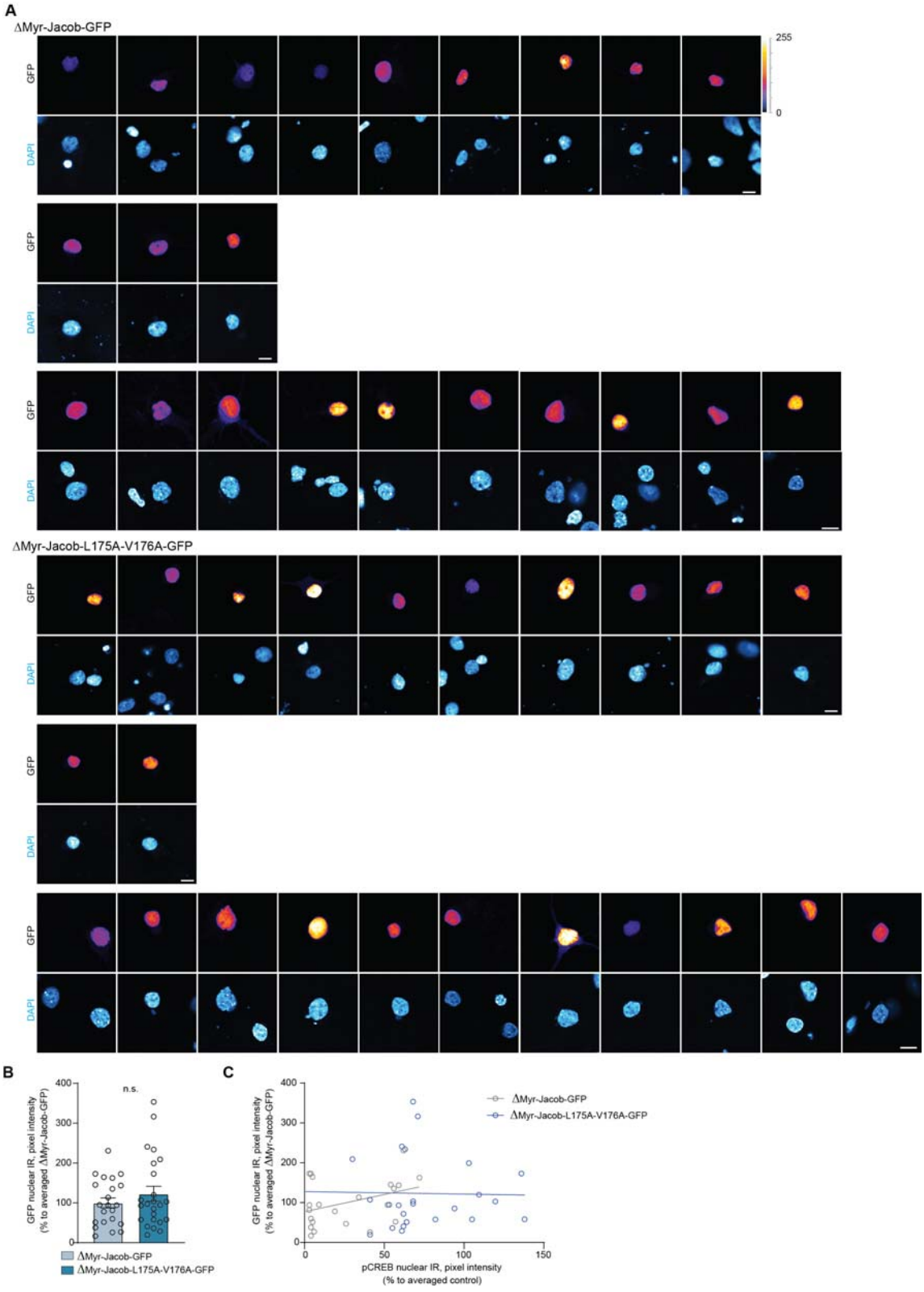
Reply: We think that there is a misunderstanding. What the reviewer is asking for is indicated in the X axis of the related graphs. The Y axis indicates the energy transfer, and the X axis indicates the expression levels of the protein fused to the acceptor divided by the expression levels of the protein fused to the donor. Thus, we took into account the expression levels of each of the proteins involved.

3- Line plots of STED images (EV2D, EV3D, E) are not very informative. Since conclusions are drawn from these data, quantitative measures of colocalization should be included.

Reply: The data showing proximity labeling between Jacob and CREB confirm their close association (see the reply for Referee #1) We therefore suggest to keep the line profiles since a statistical quantitation in this case will not add further information.

4- The amount of Jacob expressed in Fig 2(N-R) needs to be considered for results.

Reply: For obtaining data included into Fig 2N-R (Fig 2P-T in the revised version of the manuscript) we selected neurons with comparable nuclear Jacob expression levels that fitted into the same dynamic range. We include here images of the (A) GFP-positive nuclei that were analyzed, (B) the quantification of the mean fluorescence intensity of GFP. p=n.s. by Mann-Whitney test. (C) In addition we performed Spearman correlation analysis between nuclear pCREB staining intensity and GFP fluorescence signal. For both constructs we could not detect any significant correlation (p=0.3039 for ΔMyr-Jacob-GFP and p=0.4495 for ΔMyr-L175A-V176A-Jacob-GFP). Therefore, we believe that the amount of Jacob expressed does not influence the presented results. Lookup table indicates the pixel intensities from 0 to 255, scale bar is 10 μm.



5- The intent and interpretation of the Nitarsone pretreatment vs posttreatment experiments is not clear. If pretreatment works, does that mean that the Nitarsone effects are irreversible,

or does the drug not wash out? Could the sustained effects on the Jacob/LMO4/CREB complex be potentially deleterious over the long run? This section needs additional interpretation.

Reply: We have tried to make the rationale of this treatment regime clearer in the revised version of the manuscript. We have included the following sentence: In both conditions we found a rescue of pCREB levels following Nitarsone administration (Fig EV6A-C), indicating that the drug will not only prevent Jacob binding in response to Aβ-treatment but will also displace Jacob bound to LMO4 even after Aβ-induced CREB shutoff. We have not conducted experiments that suggest that Nitarsone treatment is irreversible (i.e. washout experiments) but we consider this possibility very unlikely given the results of the ITC experiments. A deleterious effect is also unlikely. The treatment with Nitarsone will be only effective when the majority of the Jacob pool is non-phosphorylated. This will only happen following sustained activation of extrasynaptic NMDAR, which only happens for longer time periods in disease.

6- In behavioral assays, statistical analyses comparing before and after treatment with nitarsone (Fig 8H-P) are needed (not just comparing across genotypes). Two-way ANOVAs should be used.

Reply: We think this is a misunderstanding. We have one time point and separate groups (treated and non-treated for each genotype). We modified the timeline schematic to make this even clearer. Nevertheless, we have used Two-way ANOVA (but not repeated measures analysis), to calculate the differences between 2 factors – genotype and treatment.

7- Overall, statistical analyses should be better described, specifically the types of tests performed for each experiment.

Reply: We have provided a summary of all respective tests with reference to the panels at the end of each Figure legend.

8- The effects of Nitarsone on excitability may be difficult to interpret. Metabolic byproducts of Nitarsone include ionic Arsenic, which can block certain K+ channels (and potentially other channels). This caveat should be mentioned in the discussion.

Reply: We don't think that this is a very likely and realistic scenario. Such studies have been performed with Arsenic trioxide, which is not a metabolite of Nitarsone. Of note, Nitarsone is metabolized at a very low rate and the concentrations of the resulting inorganic metabolites will be very low. Along these lines Nitarsone had very little effect on direct measures of intrinsic excitability in control mice.

9- In this reviewer's opinion, the paragraphs on Nitarsone in the discussion weaken the paper and should be removed. Proposing Nitarsone as a therapeutic option based on one set of experiments is not needed for the strength of this work and considerably overstates the issue (which may even be dangerous).

Reply: We understand the concern of this reviewer. On the other hand, the therapeutic potential of Nitarsone is an obvious question that we think we should address in the Discussion. We have toned down our conclusions even further in the revised version of the

manuscript. In addition, we clearly state the limitations of the present study and we believe that our conclusions are very cautious.

10-Small things- Fig 1E: There is no statistical analyses.

Reply: Following the Referee's suggestion we have included information about the statistical analysis in the Figure legend of the revised version of the manuscript.

Fig 1C: Normalized levels for pJacob/Jacob should have a value of 1 (If not, why not?).

Reply: The normalized levels for pJacob/Jacob ratios are corrected by NeuN. We normalized Jacob, pJacob, and NeuN independently (panel 1B divided by EV1C corrected by EV1E). We do not think that the second normalization of the ratios of already normalized data would be appropriate in this case. We have now provided this information in the corresponding Figure legend of the revised version of the manuscript.

FigEV6: legends are switched.

Reply: We apologize for this mistake. We have corrected this mistake in the revised version.

Fig 8A: Legend is not complete.

Reply: We apologize for this mistake and we have included the WT control in Fig. 8A.

Referee #3:

The authors report that soluble Aβ elicits cytonuclear trafficking of Jacob, which by acting as a mobile signaling hub docks a signalosome to CREB, producing transcriptional inactivation and subsequent synapse impairment and eventually loss in AD.

They also report that Nitarsone, by selectively hindering the assembly of this signalosome, restores CREB transcriptional activity. In addition, they report that Nitarsone prevents the impairment of synaptic plasticity and the cognitive decline displayed by AD mouse models.

They suggest that targeting the CREB shutoff induced by Jacob represents a therapeutic avenue against the early synaptic dysfunction in AD.

This is excellent work. I have no major suggestions to make. Hence, I recommend publication in its present form.

Only a few writing errors should be corrected, such as lack of spaces between words and between words and references.

Reply: We have corrected all typos and we want to thank the Referee for the very positive comments.

Dear Michael,

Thank you for submitting your revised manuscript to The EMBO Journal. Your manuscript has now been re-reviewed by referee #1 who appreciates the introduced changes.

I am therefore very pleased to let you know that we will accept the manuscript for publication here.

Before sending you the formal accept letter, there are just a few editorial points to sort out:

- The Data and materials availability section should be called Data Availability and should only list datasets deposited in external repositories. If none then please state This study includes no data deposited in external repositories https://www.embopress.org/page/journal/14602075/authorguide#dataavailability

- Please remove the Authors Contributions from the manuscript. The 'Author Contributions' section is replaced by the CRediT contributor roles taxonomy to specify the contributions of each author in the journal submission system. Please use the free text box in the 'author information' section of the manuscript submisssion system to provide more detailed descriptions (e.g., 'X provided intracellular Ca++ measurements in fig Y')

- Regarding the reference format - journal names should not be in bold.

- Please make sure that the funding information is consistent between online submission system and the MS file.

- Please check that there is a callout to Fig 8P

- Table S1 should be named Table EV1 - please also correct callout in text.

- Regarding Table S2 - will you correct the callout to Reagents Table

- You have 7 EV figures but can only have 5. Since the figures are quite full maybe best to move 2 figures to an appendix. Please see author guidelines for what the appendix should contain and how the appendix figures should be labelled and called out. https://www.embopress.org/page/journal/14602075/authorguide

- We need a synopsis text that contains a summary statement plus 3-5 bullet points describing the key findings of the

- We also need a synopsis image should be 550 wide by [200-400]

- The email from Gemma Navarro-Brugal bounced -please double check.

- Our publisher has also done their pre-publication check on your manuscript. When you log into the manuscript submission system you will see the file "Data Edited Manuscript file". Please take a look at the word file and the comments regarding the figure legends and respond to the issues.
Please submit a point-by-point response

That should be all - let me know if you have any questions

Best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The revised version of the manuscript is strongly improved and the authors took into account most of major concerns in a proper manner.
I still have some concern on the interpretation and rigour of the statistical analysis (see also Yu et al, Neuron 2022, 110:21-35) but I can accept the present version
For me it is now acceptable and fine to publish.

Reply to editorial comments

- The Data and materials availability section should be called Data Availability and should only list datasets deposited in external repositories. If none then please state This study includes no data deposited in external repositories
https://www.embopress.org/page/journal/14602075/authorguide#dataavailability

Done

- Please remove the Authors Contributions from the manuscript. The 'Author Contributions' section is replaced by the CRediT contributor roles taxonomy to specify the contributions of each author in the journal submission system. Please use the free text box in the 'author information' section of the manuscript submisssion system to provide more detailed descriptions (e.g., 'X provided intracellular Ca++ measurements in fig Y')

Done

- Regarding the reference format - journal names should not be in bold.

Done

- Please make sure that the funding information is consistent between online submission system and the MS file.

Done

- Please check that there is a callout to Fig 8P

We have addressed this callout

- Table S1 should be named Table EV1 - please also correct callout in text.

Done

- Regarding Table S2 - will you correct the callout to Reagents Table -

Done

- You have 7 EV figures but can only have 5. Since the figures are quite full maybe best to move 2 figures to an appendix. Please see author guidelines for what the appendix should contain and how the appendix figures should be labelled and called out.

Done. We have moved the previous Figures EV3+4 to Appendix S1+S2.

- We need a synopsis text that contains a summary statement plus 3-5 bullet points describing the key findings of the

We have included a synposis text plus 3-5 bullet points in the text

- We also need a synopsis image should be 550 wide by [200-400]

We have included a synopsis image

- The email from Gemma Navarro-Brugal bounced -please double check.

We have corrected the Email address.

- Our publisher has also done their pre-publication check on your manuscript. When you log into the manuscript submission system you will see the file "Data Edited Manuscript file". Please take a look at the word file and the comments regarding the figure legends and respond to the issues.
Please submit a point-by-point response

All requested changes were made. Also three figures were modified. In Fig. 6 panel A and E – the scale bar was added, in Fig. 3 panel K 'ns' was added, and in figure EV1, panel C, H, J ns was added.

2nd Revision - Editorial Decision **9th Dec 2022**

Dear Michael,

Thank you for submitting the revised manuscript to The EMBO Journal.

I have now looked at everything and all looks good. I am therefore very pleased to accept the MS for publication here.

Congratulations on a nice study!

best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Your manuscript will be processed for publication in the journal by EMBO Press. Manuscripts in the PDF and electronic editions of The EMBO Journal will be copy edited, and you will be provided with page proofs prior to publication. Please note that supplementary information is not included in the proofs.

You will be contacted by Wiley Author Services to complete licensing and payment information. The required 'Page Charges Authorization Form' is available here: https://www.embopress.org/pb-assets/embo-site/tej_apc.pdf - please download and complete the form and return to embopressproduction@wiley.com

EMBO Press participates in many Publish and Read agreements that allow authors to publish Open Access with reduced/no publication charges. Check your eligibility: https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/affiliation-policies-payments/index.html

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

If you have any questions, please do not hesitate to call or email the Editorial Office. Thank you for your contribution to The EMBO Journal.

** Click here to be directed to your login page: https://emboj.msubmit.net

EMBO Press Author Checklist

Corresponding Author Name: Michael R. Kreutz
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2022-112453R

USEFUL LINKS FOR COMPLETING THIS FORM
The EMBO Journal - Author Guidelines
EMBO Reports - Author Guidelines
Molecular Systems Biology - Author Guidelines
EMBO Molecular Medicine - Author Guidelines

Reporting Checklist for Life Science Articles (updated January
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions

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

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

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

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Reagents and Tools Tables, Materials and Methods

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Reagents and Tools Tables, Materials and Methods

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Reagents and Tools Tables, Materials and Methods

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Reagents and Tools Tables, Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Reagents and Tools Tables, Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Materials and Methods

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

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Yes	Materials and Methods; Table S1

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknolwedgement

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

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

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Figures and Figure legends
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Material and Methods
Include a statement about **blinding** even if no blinding was done.	Yes	Material and Methods
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Material and Methods
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figures and Figure legends; Materials and Methods

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figure and Figure legends; Materials and Methods
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure and Figure legends; Materials and Methods

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Yes	Materials and Methods
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

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

Reporting

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

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

Data Availability

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