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Peer Review File

Procognitive restoration of PV neuron plasticity in neurodevelopmental disorders

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors identify Meis2 as a novel transcriptional regulator of experience-dependent parvalbumin (PV) interneuron (IN) plasticity. Using the Cntnap2-/- mouse to model a neurodevelopmental disorder, the authors show that Meis2 overexpression remodels excitatory and inhibitory circuitry and E/I imbalance in the DG to CA3/CA2 pathway. This structural remodeling accompanies improvements in performance on a spatial and social cognitive behavioral task.

1. Meis2 is a Category 1 SFARI ASD risk gene, and, although rare missense mutations in MEIS2 are associated with autistic behavior, the relevance of Meis2 to other neurodevelopmental disorders (NDDs)- and Cntnap2-/- mice- is unclear. For example, Meis2 levels in PV-expressing (PV+) cells at baseline and the relative frequency of Meis2+/PV+ neurons are unaltered between Cntnap2+/+ and Cntnap2-/- mice (Fig. 4d). The link between Meis2 dysregulation and Cntnap2 dysregulation is unclear.

2. The authors state that viral upregulation of Tbr1, Bcl11a, Meis2, and Herc1 increases “PV IN synapses in CA2 and CA3 pyramidal neurons (Figure. 1d, Extended Data Fig. 1e)” (lines 130-131). However, the authors present PV puncta, which do not represent bona fide synapses. As a minimum, the authors must present enhanced colocalization of synaptotagmin-2 (syt2) and gephyrin to make this assertion. This claim is repeated for Fig. 2a-b and Fig. 2e-f, and colocalization of Syt2 and gephyrin is necessary to make the claim that Meis2 overexpression rescues CA3/CA2 PV IN synapses. Moreover, it is recommended that Fig. 3b and 3d be moved to Figure 2 to support the notion that Meis2 overexpression rescues CA3/CA2 PV IN synapse number.

3. It is unclear why the effect of viral upregulation of Herc1 is included in a separate graph in Extended Data Fig. 1e and not included in Fig.1d. If the authors are attempting to state that viral upregulation of all four candidate genes increases PV synapses onto CA2/CA3 neurons, then the data should be presented in the same graph.

4. The authors mention that “Meis2 is expressed at negligibly low levels in a subpopulation of hippocampal PV INs in the hippocampus” (lines 131-132). What PV IN population is expressing Meis2? Your current model overexpresses Meis2 under the S5E2 enhancer element which promotes expression of the construct in basket and chandelier PV interneurons. Is overexpression achieved in basket and chandelier CA2/CA3 PV cells, as targeted? Can overexpression, either generally or in the small population that expresses Meis2, explain why Meis2 overexpression in Cntnap2+/+ mice perform worse on OLM and social recognition compared to their control-treated counterparts?

5. The authors demonstrate enhanced transcription of Meis2 following social experience in Figure 2c-d. However, Meis2 protein levels must be assessed as protein is the functional readout.

6. In Extended Data Fig. 2, validation of AAV-S5E2-Meis2-P2A-nlsdTomato and AAV-S5E2-dTom-P2A-nlsdTomato is

performed, demonstrating ~70% transfection efficiency in PV+ neurons. What is the transfection efficiency of the other XPG-overexpressing viruses (Tbr1, Bcl11a, and Herc1)? Moreover, the authors should also present histological evidence of Meis2, Tbr1, Bcl11, and Herc1 overexpression in PV+ neurons in Fig. 1d.

7. The authors state that "Ex vivo whole cell patch-clamp recordings from PV INs and CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current in CA2 PNs compared to Cntnap2+/+ mice… (Fig. 3a, Extended Data Fig. 3a)" (lines 171-173), yet this reviewer can find no inclusion of sEPSC recordings from PV INs.

8. The author's claim that their analysis of "Meis2 and Tbr1 suggests that these two XPGs independently and differentially contribute to experience-dependent PV IN plasticity" (lines 236-237); however, the authors provide incomplete evidence for this case. 1) No histological evidence is provided to confirm Tbr1 was indeed overexpressed. 2) Tbr1 overexpression was performed in only Cntnap2+/+ mice, and it is fundamentally important to determine if Tbr1 overexpression rectifies synaptic properties in Cntnap2-/- mice, as Meis2 overexpression produced differential responses in Cntnap2+/+ and Cntnap2-/- mice. 3) Does Tbr1 overexpression alter PV s/mEPSC frequency and amplitude?

9. Meis2 overexpression in Cntnap2-/- mice does not restore PV IN mEPSC frequency but is sufficient to rescue PV IN mEPSC amplitude (Fig. 3e) and PV IN AP spiking (Fig. 3i). As Meis2 is a transcription factor, it will be helpful to know which of its target genes are transcriptionally enhanced. Moreover, is there transcriptional convergence of other XPGs (e.g. Tbr1), as the data presented suggest enhancing either XPG is sufficient to restore developmental deficits?

10. Meis2 overexpression in Cntnap2+/+ mice appears to worsen performance on the OLM task (Fig. 4c) yet the authors do not comment on this. Similarly, Meis2 overexpression exacerbates performance in social recognition (Fig. 4f), and the authors provide an insufficient explanation ("when GABAergic inhibition is intact increasing MEIS2 dosage does not enhance cognition" [lines 260-261]) for why Meis2 enhancement exacerbates performance on the OLM and social discrimination task. What do the authors mean by intact GABAergic inhibition? Cntnap2+/+ mice have statistically indistinguishable CA2 PN sIPSC amplitude and CA2 PN mIPSC frequency and amplitude from Cntnap2-/- mice. Furthermore, Meis2 enhancement in Cntnap2+/+ mice caused 1 out of the 6 mice to seize (Extended Figure 8a-c); if GABAergic inhibition is indeed intact, what precipitated this seizure?

11. The authors should describe why Cal-Light is being conducted (over other calcium sensors) and the technical modifications implemented to test PV activation in their model. What is the transfection efficiency of the three employed viruses, and how might poor transfection affect the interpretation put forth? Moreover, the authors provide no representative images of the number of active cells in control/Meis2-boosted Cntnap2+/+ and Cntnap2-/- mice during the reactivation phase. The image of cFOS+/GFP+ cells (Fig. 4h) is not nearly sufficient.

12. It is not specified why the authors chose to test the effects of boosting Meis2 levels on seizure generation other than the simple fact that Cntnap2-/- mice exhibit spontaneous seizures. According to the SFARI database, MEIS2 mutations are not associated with epilepsy, thus the relevance of this experiment to cognitive restoration needs to be provided.

13. The authors claim that overexpressing Meis2 in PV+ neurons in the DG of Cntnap2-/- mice is sufficient to suppress seizures. However, the data presented are unconvincing. It is surprising that overexpressing Meis2 in an extremely sparse cell population is able to abate seizure generation as measured via ECoG. Moreover, a single control treated Cntnap2-/- mouse appears to be driving this effect (Fig. 5c). To confirm the veracity of this finding, the authors should increase the sample size in Fig. 5 and utilize an acute seizure model (e.g. PTZ, kainic acid, etc.) to determine if Meis2 overexpression suppresses seizures.

Minor Concerns

1. In the introduction, the authors state they are testing a "prototypical NDD risk model" (line 99). What makes the Cntnap2-/- mouse a prototypical NDD risk model compared to Fmr1-KO, Tsc1+/-, Tsc2+/-, or Mecp2-/-, for example?

2. Supplementary Table 1 needs to be organized between significantly upregulated, downregulated, and unchanged genes. Moreover, the authors state "18 of 67 ASD genes encode histone methyltransferase, histone demethylases, ATP-dependent chromatin remolding factors, transcription factors and coactivators" (lines 114-116), yet these genes are not highlighted in Supplementary Table 1. In its current state, it is unclear which genes are upregulated, downregulated, or unchanged or are associated with a particular functon(e.g. histone demethylation).

3. "Significantly upregulated genes included 67 Category 1 ASD SFARI genes" (lines 110-111), however, in Figure 1c, only 47 genes are displayed as Category 1 ASD SFARI genes. Why were these 47 genes presented, and what are the other 20 Category 1 ASD SFARI genes? If select genes were chosen to be presented, why were these genes decided upon?

4. The following change should be made to lines 171-174, as 1) the current phrasing is ambiguous as to which measure (frequency or amplitude) changes on PV interneurons, 2) the incorrect Figures are referenced in the text, and 3) Meis2 augmentation does not affect PV IN mEPSC frequency: "Ex vivo whole cell patch-clamp recordings from CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current frequency and amplitude in CA2 PNs compared to Cntnap2+/+ mice, and boosting Meis2 in PV INs reduced sEPSC frequency and amplitude in Cntnap2-/- and Cntnap2+/+ mice (Fig. 3a, Extended Data Fig. 3a)" (lines 171-174).

5. The labels for the x-axis in Figure 4 should be present throughout the entire Figure (e.g. Fig. 4c has no x-axis, but the x-axis labels are present in Fig. 4e).

6. Please make sure all abbreviations used in the figures are defined.

Referee #2

(Remarks to the Author)
Summary of the key results: This study uncovered experience-dependent plasticity genes (XPGs) that are essential for PV-IN function and are part of genetic risk factors in the context of adjustment of inhibition. Shih et al. found Meis2 as a key XPG and studied the impact of Meis2 expression in restoring hippocampal activity. Using viral vectors, electrophysiology and behavioural assessments, the authors reveal that restoring PV-IN plasticity in the adult hippocampus is sufficient to reverse developmental deficits in a mouse model of autism.

Originality and significance: Whilst this study is of significance and brings substantial insights into neuronal plasticity in the adult brain, the general concept of targeting activity-dependent molecules and manipulating PV-INs to reverse developmental impairments is not new.

Selimbeyoglu A, Kim CK, Inoue M, Lee SY, Hong ASO, Kauvar I, Ramakrishnan C, Fenno LE, Davidson TJ, Wright M, Deisseroth K. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Sci Transl Med. 2017 Aug 2;9(401):eaah6733. doi: 10.1126/scitranslmed.aah6733. PMID: 28768803; PMCID: PMC5723386.

Yap EL, Pettit NL, Davis CP, Nagy MA, Harmin DA, Golden E, Dagliyan O, Lin C, Rudolph S, Sharma N, Griffith EC, Harvey CD, Greenberg ME. Bidirectional perisomatic inhibitory plasticity of a Fos neuronal network. Nature. 2021 Feb;590(7844):115-121. doi: 10.1038/s41586-020-3031-0. Epub 2020 Dec 9. PMID: 33299180; PMCID: PMC7864877.

Data & methodology: The major limitation of the study is the use of a viral construct that is not entirely specific to PV-INs (Extended Fig 2a, b). Meis2 is poorly expressed in PVINs, compared to pyramidal cells. Therefore, the impact of Meis2 expression in pyramidal cells on influencing hippocampal circuits cannot be overlooked. To validate the conclusion that specific Meis2 expression in PV-INs underlies reversal of developmental deficits in the Cntnap2 KO mice, further analysis of the distribution and expression patterns of Meis2, as well as the physiological properties of the pyramidal cells, is necessary.

Statistics and biological replicates: Some statistics are missing (e.g. Fig.5), or inconsistent (e.g. Fig2d, top (no statistical differences between groups) vs bottom panel (statistical differences between groups). Certain analyses require a higher number of biological replicates (e.g. Extended data fig1 b: n=2; Extended data fig2 b: n=3; Extended data fig7 d: n=3 for social behaviour) to enable more robust statistical evaluation.

The seizure analysis (Fig. 5) currently appears to lack sufficient strength to support the statement on line 290 regarding the boosting of Meis2 to efficiently suppress seizures in the Cntnap2 KO mice. Only the number of seizures/day changes between conditions. The statistics (Fig 5c) appear to be driven by a single mouse sitting at 20+ seizures a day (which is likely an outlier), compared to fewer than 2 seizures for the remaining KO mice. How does the occurrence of seizures compare to what has been reported in the literature for this model? In addition, statistical analysis for Fig.5d is required (e.g. Chi Square test).

The authors did not report any sex differences in the KO mice compared to WT. On lines 853-855, it is mentioned that “if no statistical difference or interaction between sex were observed, then mice were grouped…”. This is surprising. How to compare when the total n for some experiments is <5 (e.g. Fig3, in particular for the behaviour (fig 4)? In particular, please check Extended data fig.7d in which there are only n=3 mice for the behavioural assessment. For some reason, the histograms do not show the same n values (i.e. nose-nose, nose to center, nose to rear and total interactions), despite representing data from the same mice.

Conclusions:

-The major concern in the present study is the experimental approach, using a virus that is not entirely specific to PV-INs (line 162-163). Extended Data Fig.2 is crucial as it reveals the quality of the viral infection and type of cells that are affected by the infection. Presented results do not support the statement that selective boosting of Meis2 expression in CA3/CA2 PV INs reverses developmental deficits in the hippocampus and associated behaviour. In fact, only 70% of infected cells seem to be PV INs, with 30% likely to be pyramidal cells, which express high levels of Meis2 (cell in the middle in Extended Data Fig.2a (arrowhead should be unfilled), Extended data Fig. 2b & Fig 2c, see Meis2 expression in non-PV cells vs PV cells). The authors report that Meis2 is expressed at negligibly low levels in a subpopulation of PV interneurons. What is the proportion of Meis2-expressing PV and non-PV cells? Do they all have Meis2 protein? Unfortunately, there is no comparative analysis of Meis2 expression in PV-INs vs pyramidal cells (proportion of expressing cells and mean intensity levels). What is the percentage of PV+ cells infected with AAV-S5E2-Meis2-nls tdtomato? It seems that barely 25% are infected according to Extended Data Fig.2a. In that context, data does not support the conclusion that specific Meis2 expression in PV-INs restores deficits in the Cntnap2 KO mice.

-No difference in Meis2 levels in PV cells control vs Cntnap2 KO is reported (Fig.2d top &bottom). How can Meis2 support the beneficial effect of the Meis2 viral infection in that context?

-Pyramidal cells, which express high levels of Meis2, are likely the main cells responding to the boost in Meis2 expression to

trigger a cascade of events involving PV-INs. One could argue that the restoration of perisomatic synapses observed in CA2 in Fig.2 e,f could be an effect of the AAV-S5E2-Meis2-nls tdtomato expression in pyramidal cells? Authors should prove the specificity and discuss this important validation point to solidify results.

- The statement on line 191-192: “Boosting Meis2 in PV INs attenuated…” is an overstatement as pyramidal cells are likely to be affected as well. Evidence with physiological and immunological assessments in pyramidal cells in control and Meis2 viral infection in control and Cntnap2 KO mice are necessary (e.g. Fig.3g: DG inputs principally target CA2 pyramidal cells).

- The reason why the authors selected Tbr1 over other key genes to study how they influence PV INs function is not well introduced. Is Tbr1 expressed in all PV INs? In pyramidal cells? In fact, the impact of Tbr1 on hippocampal function is less impressive than the ones observed with Meis2. I am not sure that the current data supports the conclusions from line 233. I recommend this section to be strengthened with more compelling results.

- Absence of qualitative differences between the WT and Cntnap2 KO mice (e.g. social interactions (Extended Data Fig. 7d) and epilepsy (Fig.5; Extended Data Fig. 8)) is surprising. Social alterations represent a key feature of the Cntnap2 KO mice (Penagarikano et al 2011 Cell).

Suggested improvements:
-The conclusions of the study would be significantly strengthened by showing that specific Meis2 expression in PV-INs supports restoration of developmental deficits (e.g. behaviour) in other (genetic and/or non-genetic) models of NDDs (e.g. shank3 ko, valproate acid model etc…).

- A discussion point regarding potential compensatory mechanisms from other cells that are affected by the Meis2 viral injection (i.e. pyramidal cells) should be added in the manuscript. Considering that Meis2 maintains intrinsic excitability and terminal arborization in neurons (line 136), it is likely that Meis2 expression shapes pyramidal cell activity and morphology. This point must be addressed, as it is essential for the conclusion of the manuscript (i.e. Direct vs indirect regulation of the PV-IN potential). An analysis of the physiological properties of pyramidal cells under the different experimental conditions is required to confirm that they are not affected, and do not directly contribute to the phenotype observed.

-The MFF of the PV INs is lower in the KO and restored by Meis2. This could be synaptically-driven by pyramidal cells, as their firing properties are boosted by Meis2 expression (see extended data fig.3g, right top vs bottom) and/or by PV INs receiving more glutamatergic inputs (Fig. 3e). Recordings as in Fig3i under synaptic blockers could confirm that the different physiological properties between the experimental groups are solely due to different intrinsic properties of PV INs following Meis2 addition.

-What are the target genes of Meis2 in PVIN and pyramidal cells? To understand how Meis2 works, it would be beneficial to include some supporting Cut&Run/ Cut&Tag data.

-In the discussion section, the authors mention that Meis2 expression is virtually absent in the MGE, suggesting that hippocampal PV-INs expressing Meis2 are likely derived from another embryonic region. What about the preoptic area which can represent another source of hippocampal PV interneurons?

References: Overall, references were adequately cited. I could not find any report of Meis2 levels for Reference 37.

Clarity and context: Whilst the manuscript effectively outlines the contribution of PV-IN plasticity in shaping the adult brain, it is already well established that PV IN dysfunction represents a hallmark of NDDs. This study uncovered key molecules that are modified upon experience and are linked to NDDs. The justification for focusing on Meis2 and Tbr1 rather than other XPGs is weak. Meis2 is sparsely expressed (line 132), compared to other genes detected (Fig1.c). Whilst the authors claim that specific increase in Meis2 in PV IN restore deficits in the Cntnap2 KO mice, the fact that Meis2 is poorly expressed in PV IN (few cells positive and weak expression) compared to other hippocampal cells must be discussed.
The authors elaborated on the impact of PV INs on blanket inhibition and selective inhibition of distinct pyramidal cells (line 349-350) as a potential mechanism to remap network dynamics. However, this raises the question as to how boosting Meis2 expression specifically in a subset of PV INs would be enough to reshape the entire hippocampal circuitry and behaviour. Further discussion is required.

Minor comments:
- Line 207-208: How iLTD is different across groups? Any statistics for that?
Could it be possible to add a table of the intrinsic properties of patched cells (e.g latency, AP properties…). This will prove informative.
- Line 366: may be, not maybe. And 368: regulate, no regulating.
- Line 370: Please briefly clarify in this sentence the overlapping vs distinct effects of Tbr1 and Meis2.
- Why presenting similar data in fig 3h (IPSC LTD, left) and extended data fig 4b (IPSC ltd, middle)? In addition, the figures only show +/+ ctl conditions. It would be informative to see traces from all conditions in fig 3h left.
- Lines 342-343: Is Meis2 also expressed in a subset of cortical PV INs? What is the percentage there?
- Lines 373-374: The idea needs further clarification. What are the possible explanations or mechanisms behind it?
- From line 374, the authors uncovered hundreds of genes that are altered following experience in PV INs. However, deciphering combinatorial “codes” and understanding their functional relevance remains inaccessible, and this limitation

should be explicitly stated.

Referee #3

(Remarks to the Author)
This manuscript, entitled, “Pro-cognitive restoration of experience-dependent parvalbumin inhibitory neuron plasticity in neurodevelopmental disorders,” by Yu-Tzu Shih and others examines and tests the molecular and circuit plasticity mechanisms underlying experience-dependent plasticity of parvalbumin-expressing interneurons (PV IN). The authors search for transcriptional regulators that are linked to enhanced mossy fiber-PV transmission, a correlate for experienced-dependent PV In plasticity. They identify several candidates and take a closer look at one in particular, Meis2. They use virally-mediated Meis2 upregulation in the background of a neurodevelopmental mouse model (Cntnap2 -\-). With optogenetics and ex-vivo physiology they examine the consequence of Meis2 up-regulation on transmission, plasticity and intrinsic properties of synapses on CA2 pyramidal neurons. Furthermore, they test the hypothesis that up-regulation of Meis2 can rescue social cognitive deficits in the NDD mouse model.

The work in this manuscript is highly original. The genetic screen is an invaluable contribution to the field, and these findings may potentially have a lasting impact on our understanding of experience-induced plasticity. It is more in the investigation of the mechanism that the experimental strategies and interpretation of the data have potential and serious confounds. However, the authors have discovered something substantial and important, and they demonstrate that this has unquestionable importance for neurodevelopmental disorders. It is more weaknesses, in my opinion in the experiments that they use to fully understand the mechanism that this paper falls short in its current state.

Major concerns:
1)
The authors use the Cntnap 2 -\- mouse model to examine how up-regulation of Meis2 alters synaptic transmission, plasticity and intrinsic properties in hippocampal area CA2. Because of the importance of hippocampal area CA2 in social memory and established link with numerous psychiatric disorders, this makes some sense. The authors focus their attention on the mossy fiber pathway (MFP) input to PV INs in areas CA2/CA3. While this connection is important, I think that the authors need to expand their perspective and consider the other inputs. In addition to the MFP, the Schaeffer collateral inputs from CA3 synapse onto PV INs in area CA2. Furthermore, CA3 SC inputs also synapse onto CA2 Pyramidal cells. To complicate things more, CA2 PCs projects back to both CA3 interneurons and CA3 pyramidal cells (see Boehringer, et al, 2017). (These are just the intra-hippocampal inputs, there are also hypothalamic and cortical synapses). My point here is that the spontaneous EPSCs in CA2 are coming from many different regions, not just the MFS. And likewise, the spontaneous IPSCs are from inhibitory synapses of neurons that are being recruited by numerous excitatory inputs. Care must be taken to not over-interpret the results of figure three. An increase of sEPSC frequency could correspond to hyperexcitability at an input… or an increase in synapse number. If there is an increase in synapse number, which synapse of the many to choose from? Furthermore, and increase in amplitude becomes even tougher to interpret, given the size of the pyramidal cells dendritic arbor and massive attenuation in the apical dendrite. Could in increase in amplitude indicate an increase in proximal synapses? An increase in AMPA receptors at the post-synaptic site? Evoking transmission and performing input/output curves with electrical stimulation before and after blocking GABA receptors for the different inputs would be a straightforward way of determining which dendritic compartment and class of interneurons are being involved.

The situation for miniature EPSCs and IPSCs is simpler, as the feed-forward aspect of the local circuitry is no longer at play. I find it to be really fascinating how there appears to be no difference at all in the mEPSC amplitude, yet a substantial difference for the sEPCS. Could the authors comment on this? It may be helpful to measure the sEPCs in the presence of GABA receptor blockers. The interpretation that the local inhibitory network or feedforward inhibition may be shunting excitation could be examined this way.

The best way to really understand the PV IN – CA2 synapse would be to perform paired recordings and not rely on multi-synaptic methods of excitation. However, given the size of CA2 and the complexity involved in these experiments, I understand why the authors have relied on different methods.

The optogenetics in figure 3 and extended data figure 4 needs to be more carefully interpreted. Channelrhodopsin is expressed in the mossy fiber terminals, and synaptic transmission is being evoked by light stimulation. Transmission will occur by a combination of methods: 1) depolarization of mossy fiber axons, resulting in action potentials and vesicle release, and 2) direct depolarization of synaptic terminals. These two methods will have different dynamics and dependance on light intensity. It is not completely sound to interpret paired-pulse ratios with only one intensity of light stimulation. There needs to be an input-output control performed with and without TTX to demonstrate that the light intensity used is due to action-potentials only. The data that is there might be fine, they authors need to provide the evidence that the experiment was done properly. The pulse is short-ish (1 ms) and the light is over the hilus, but confirming with TTX would be confirmatory.

The IPSC LTD experiment is very nicely done (panel h). What plasticity mechanism is this? Delta-opioid mediated at the inhibitory terminals onto the CA2 pyramidal cells? This is consistent with what is seen in the PPR results in extended figure 4. I think the authors could comment or explore a little bit more.

The whole-cell recording of the PV INs in area CA2 are beautiful. Very nice work. Why not analyze the shape of the action potential? If resting membrane potential was not changed, what about membrane resistance? AHP?

2) The behavior seems very cursory to me.

There is a lot of evidence indicating that novel object location is unchanged when hippocampal area CA2 is lesions, or when inhibitory synaptic plasticity is altered. Are the authors now interested in the DG-CA3 circuit?? Also, in panel a, the differences in viral expression and localization are striking and put into question the results.

Why is the discrimination index not shown for the social memory testing? This is commonly measured and plotted. Why not have habituation as well? Why only 5 minutes between tests? The time interval chosen is pushing the limit of working memory/ hippocampal-dependent memory. At least 10 minutes would be better… or an hour. This seems very minimal for such an important demonstration of a rescue of a neurodevelopmental mouse model during adulthood.

3) I do not like at all the use of the Cal-Light system without the proper demonstration that the dynamic calcium levels of CA2 pyramidal cells during social exploration are relevant for this system. Where is the negative control? Where is the positive control? There is no prior evidence that social information is encoded in area CA2, more ventral CA1. The immunostaining in panel h is very saturated, and there is no indication of what cell types are labeled (RGS14 or PV staining).

To me, the epilepsy experiments look very promising but are also a bit preliminary. Why not look at seizure threshold, or length with stimulation (trans-cornial or septal stimulation?). Pharmacological stimulation? I could probably be convinced if there are good arguments. Furthermore, for the Meis2 animals, why not examine epilepsy-related histological markers?

Minor problems:
1) In figure 2C, The images are too saturated and zoomed in to be useful in assessing location in the hippocampal region, or the overall phenomenon that the authors are trying to illustrate. From what I can tell, it appears that Meis2 is not expressed in PV cells, but rather, in CA2 pyramidal cells? Considering that the Meis2 gene was identified following isolation from PV-Cre; Rpl22HA.HA mice. Could the authors comment on this?
2) The cartoons in Figure 3 (all panels) absolutely must be corrected!! The MFS does not synapse onto distal apical dendrites of CA2!

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)
This paper is vastly improved, and the authors have addressed many methodological concerns. Some concerns remain:

-Sample sizes remain low in some studies. In most cases, the authors can get away with a low sample size of 4-5, as the effect sizes are large and/or the variance is low. However, there are a few examples when a low sample size is worrisome. For example, the negative data in Figure 1g could be a consequence of under sampling, as the sample size is low and the variability is relatively high compared to other measurements. This lack of effect could easily be a Type II error that very much changes the interpretation of the data (e.g. – suggesting that the Meis2 effect is exclusively driven by its upregulation in PV+ interneurons rather than also through its upregulation in pyramidal neurons).

-Given the emphasis on the Meis2-driven upregulation of PV interneuron synapses onto CA3 and CA2 pyramidal neurons, -- if feasible-- it would be informative to perform triple-labeling studies to include a neuronal marker and/or triple-labeling studies including a dendritic marker to more incisively define sites of PV synaptogenesis. Alternatively, one could conceivably use Td-tomato-labeled PNs instead of neuronal/dendritic markers to examine anatomical targets of enhanced PV synaptogenesis.

The importance of PV interneurons in experience-dependent plasticity, cognition, and seizures is well established. This study adds novelty by showing, in a genetic model with dysregulated Meis2 expression, that rescue of experience-dependent Meis2 upregulation in PV interneurons can reinstate these important functions of parvalbumin interneurons.

Referee #2

(Remarks to the Author)
This manuscript presents a comprehensive and experimentally rigorous investigation into experience-dependent plasticity genes (XPGs) and their role in parvalbumin interneuron (PV-IN) function in the adult hippocampus. The authors identify Meis2 as a key regulator and demonstrate, through a combination of viral targeting strategies, electrophysiology, and behavioural assays, that restoring PV-IN plasticity in adulthood can reverse circuit and behavioural deficits in a Cntnap2 knockout mouse model. That said, as previously noted, the broader conceptual framework of modulating inhibitory circuitry to rescue neurodevelopmental phenotypes is not entirely new. The novelty here lies in the identification of Meis2 and the demonstration of its cell-autonomous role in PV-IN plasticity.

Overall, the revised manuscript has been substantially strengthened. The authors have clearly invested significant effort in addressing my concerns, and the additional experiments provide strong support for their central conclusions. A major strength of the study is the breadth and depth of experimental validation. In particular, the authors show that Meis2

expression in PV-INs is sufficient to restore excitatory/inhibitory balance, hippocampal network activity (including sharp-wave ripples), and behaviour. They implemented a genetic-temporal strategy (PV-Cre; AAV-DIO-Meis2) to restrict expression to adult CA3/CA2 PV interneurons and provided appropriate controls to validate the specificity of the S5E2 enhancer-based viral approach. In addition, seizure analyses have been strengthened through increased sample sizes and improved statistical rigor. The identification of putative downstream targets of MEIS2, together with the XPG portal, represents a valuable resource for the community.
These additions significantly increase confidence that the observed phenotypes are primarily driven by PV-IN-specific mechanisms rather than off-target effects. The study provides important insights into cell-autonomous regulators of experience-dependent PV-IN plasticity. This work advances the field by identifying a molecular regulator linking experience-dependent transcriptional programs to circuit function.

I suggest one minor point that would further improve the discussion. The manuscript highlights that Meis2 is expressed at low baseline levels in PV-INs but is induced by experience. Given its relatively sparse expression compared to pyramidal neurons, it would be useful to more clearly explain how modulation within a subset of PV-INs can produce large-scale network and behavioural effects.

Conclusion: This is a strong and substantially improved manuscript. I would like to commend the authors for the significant effort invested in addressing the reviewers' concerns and for the thorough additional experiments. Overall, the work provides compelling evidence that Meis2 acts as a cell-autonomous regulator of PV-IN plasticity in the adult hippocampus. I support publication after minor revision addressing the point above.

Referee #3

(Remarks to the Author)
In this exceptional work, the authors use an original and effective screen to identify the homeobox gene Meis221 as a regulator of experience dependent plasticity in areas CA2 and CA3 of the hippocampus. THe authors then demonstrate in a heroic show of force how this gene can be harnessed to rescue fast spiking PV cell properties and corresponding cognitive deficits in neurodevelopmental disorders in adulthood.

I congratulate the authors on a remarkable contribution to the field. All of my comments have been thoughtfully addressed. This manuscript is comprehensive and the new experiments, while extensive, are very well presented and performed. The manuscript is well-written.

[redacted]

Referees' comments:

Referee #1 (Remarks to the Author):

The authors identify Meis2 as a novel transcriptional regulator of experience-dependent parvalbumin (PV) interneuron (IN) plasticity. Using the Cntnap2-/- mouse to model a neurodevelopmental disorder, the authors show that Meis2 overexpression remodels excitatory and inhibitory circuitry and E/I imbalance in the DG to CA3/CA2 pathway. This structural remodeling accompanies improvements in performance on a spatial and social cognitive behavioral task.

1. Meis2 is a Category 1 SFARI ASD risk gene, and, although rare missense mutations in MEIS2 are associated with autistic behavior, the relevance of Meis2 to other neurodevelopmental disorders (NDDs)- and Cntnap2-/- mice- is unclear. For example, Meis2 levels in PV-expressing (PV+) cells at baseline and the relative frequency of Meis2+/PV+ neurons are unaltered between Cntnap2+/+ and Cntnap2-/- mice (Fig. 4d). The link between Meis2 dysregulation and Cntnap2 dysregulation is unclear.

We chose the Cntnap2 KO model because it exhibits a plethora of developmental deficits and not just a reduction in PV INs and PV IN dysfunction. As such, this model allows us to test the significance of targeting experience-dependent PV IN plasticity as a generalizable pro-cognitive, anti-epileptic intervention for NDDs.

In our manuscript, we convey this rationale twice and we will do a better job conveying it even more clearly in the revised manuscript.

Line 137: Since many NDDs including ASD are characterized by a plethora of developmental deficits, intellectual disability, seizures and impaired social cognition, we selected *Cntnap2* KO (*Cntnap2*$^{-/-}$) mice to test our hypothesis. Specifically, *Cntnap2*$^{-/-}$mice exhibit an array of developmental deficits including loss of hippocampal and cortical PV INs, social and spatial cognitive impairments and seizures by around 4-6 months of age[40-43].

Line 322: To test the hypothesis that loss of experience-dependent PV IN plasticity in CA3/CA2 is a substrate for cognitive impairments and seizures in NDDs, we chose the *Cntnap2* KO model of NDD risk. This was not to model a specific NDD, but because it exhibits a plethora of developmental brain-wide deficits including neuronal migration, myelination, neuronal excitability, loss of GABAergic inhibition and PV INs, cognitive impairments and seizures[40-43].

2. The authors state that viral upregulation of Tbr1, Bcl11a, Meis2, and Herc1 increases "PV IN synapses in CA2 and CA3 pyramidal neurons (Figure. 1d, Extended Data Fig. 1e)" (lines 130-131). However, the authors present PV puncta, which do not represent bona fide synapses. As a minimum, the authors must present enhanced colocalization of synaptotagmin-2 (syt2) and gephyrin to make this assertion. This claim is repeated for Fig. 2a-b and Fig. 2e-f, and colocalization of Syt2 and gephyrin is necessary to make the claim that Meis2 overexpression rescues CA3/CA2 PV IN synapses. Moreover, it is recommended that Fig. 3b and 3d be moved to Figure 2 to support the notion that Meis2 overexpression rescues CA3/CA2 PV IN synapse number.

The formal way to demonstrate that Meis2 overexpression in PV INs rescues inhibitory synapses onto CA2 pyramidal cells is to perform whole-cell patch clamp electrophysiological recordings of spontaneous and mini IPSCs and record mossy fiber-evoked IPSCs in CA2 neurons which we have done in Figure 3. Anatomical analyses are important to directly visualize changes in distribution of PV synapses on CA2 neurons and in prior work, we show that changes in PV puncta are reflected in changes in frequency of mIPSCs (Shih et al, Neuron 2023). As requested, we will perform Syt2 and gephyrin analyses to capture changes in distribution of PV IN synapses onto CA2 neurons.

3. It is unclear why the effect of viral upregulation of Herc1 is included in a separate graph in Extended Data Fig. 1e and not included in Fig.1d. If the authors are attempting to state that viral upregulation of all four candidate genes increases PV synapses onto CA2/CA3 neurons, then the data should be presented in the same graph.

We will reformat accordingly.

4. The authors mention that "Meis2 is expressed at negligibly low levels in a subpopulation of hippocampal PV INs in the hippocampus" (lines 131-132). What PV IN population is expressing Meis2? Your current model overexpresses Meis2 under the S5E2 enhancer element which promotes expression of the construct in basket and chandelier PV interneurons. Is overexpression achieved in basket and chandelier CA2/CA3 PV cells, as targeted? Can overexpression, either generally or in the small population that expresses Meis2, explain why Meis2 overexpression in Cntnap2+/+ mice perform worse on OLM and social recognition compared to their control-treated counterparts?

Please take a look at Supplementary tables (GSE142546_tpm_all-simplified) for Reference 37: Que et al., *Nature communications* **12**, 108 (2021). https://doi.org/10.1038/s41467-020-20328-4. In their RNA sequencing data, Meis2 is mainly expressed in basket cells. Note, Meis2 is expressed at negligibly low levels in a subpopulation of vertical basket cells.

Expression of Meis2 under S5E2 enhancer element targets all PV INs including basket cells and chandelier cells. Currently, it is impossible to dissociate between these subtypes of PV INs (let alone different subtypes of basket cells) and our efforts reflect the state-of- the art tools. Even the proposed use of PV-Cre mice to defend our claim that boosting Meis2 in PV INs rescues E-I balance in Cntnap2 mice is limited by an inability to distinguish between PV subtypes. Caveats notwithstanding, our finding has the potential to advance the field and as you allude to, stimulate investigation of roles of different CA3/CA2 PV IN subtypes in social cognition.

Boosting Meis2 in PV INs of Cntnap2+/+ mice increases PV IN synapses onto CA2, enhances PV IN dependent synaptic plasticity and increases PV IN intrinsic excitability. It is possible that network changes during the two weeks following Meis2 overexpression in PV INs results in an increase in sEPSC frequency that impairs cognition. Indeed, Cntnap2 KO mice exhibit a large increase in sEPSC frequency compared to Cntnap2+/+ mice and boosting Meis2 in PV INs of Cntnap2+/+ mice shifts the line plot for sEPSC frequency in the same direction as that seen for Cntnap2 KO mice.

We have generated PV-Cre; Cntnap2 KO bigenic mice and are engineering a Cre-ON AAV-DIO-Meis2 virus to use in combination with these mice to boost Meis2 exclusively in CA3/CA2 PV INs. We will characterize changes and extent of rescue of inhibitory and excitatory synaptic transmission in DG-CA2 of Cntnap2 KO and WT littermates (4 groups).

5. The authors demonstrate enhanced transcription of Meis2 following social experience in Figure 2c-d. However, Meis2 protein levels must be assessed as protein is the functional readout.

Despite our best efforts, we cannot trust commercially available Meis2 antibodies. We will continue trying antibodies. Please note that we discovered Meis2 in addition to other XPGs through analysis of the PV IN translatome and not transcriptome. Thus, our analysis suggests that Meis2 protein levels are dynamically controlled in PV INs through experience-dep regulation of the translatome.

6. In Extended Data Fig. 2, validation of AAV-S5E2-Meis2-P2A-nlsdTomato and AAV-S5E2-dTom-P2A-nlsdTomato is performed, demonstrating ~70% transfection efficiency in PV+ neurons. What is the transfection efficiency of the other XPG-overexpressing viruses (Tbr1, Bcl11a, and Herc1)? Moreover, the authors should also present histological evidence of Meis2, Tbr1, Bcl11, and Herc1 overexpression in PV+ neurons in Fig. 1d.

We will furnish these analyses.

7. The authors state that "Ex vivo whole cell patch-clamp recordings from PV INs and CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current in CA2 PNs compared to Cntnap2+/+ mice… (Fig. 3a, Extended Data Fig. 3a)" (lines 171-173), yet this reviewer can find no inclusion of sEPSC recordings from PV INs.

Yes, as stated, we patched PV INs and CA2 PNs and we saw an increase in sEPSC frequency in CA2 PNs. We will record sEPSCs onto PV INs.

8. The author's claim that their analysis of "Meis2 and Tbr1 suggests that these two XPGs independently and differentially contribute to experience-dependent PV IN plasticity" (lines 236-237); however, the authors provide

incomplete evidence for this case. 1) No histological evidence is provided to confirm Tbr1 was indeed overexpressed. 2) Tbr1 overexpression was performed in only Cntnap2+/+ mice, and it is fundamentally important to determine if Tbr1 overexpression rectifies synaptic properties in Cntnap2-/- mice, as Meis2 overexpression produced differential responses in Cntnap2+/+ and Cntnap2-/- mice. 3) Does Tbr1 overexpression alter PV s/mEPSC frequency and amplitude?

The primary motivation behind performing additional evidence for other XPGs is to demonstrate and contrast the effects of boosting different XPGs in PV INs on PV IN properties (synapse number, synapse physiology, synaptic plasticity). The use of Cntnap2+/+ mice is justified for this purpose. The limited effect of boosting Tbr1 in PV INs of Cntnap2+/+ mice compared to that seen with Meis2 motivated us to focus on Meis2 for the rescue experiments. We hope that our publicly shared XPG dataset and preliminary characterization of other XPGs will facilitate investigation of these XPGs. However, it is simply beyond the scope of this study to test these different XPGs for their pro-cognitive restorative potential in Cntnap2 KO mice as we have done for Meis2.

If the reviewers would like us to remove the data for other XPGs, we are happy to oblige. We are happy to provide this data for broad access to the neuroscience community on our lab website.

9. Meis2 overexpression in Cntnap2-/- mice does not restore PV IN mEPSC frequency but is sufficient to rescue PV IN mEPSC amplitude (Fig. 3e) and PV IN AP spiking (Fig. 3i). As Meis2 is a transcription factor, it will be helpful to know which of its target genes are transcriptionally enhanced. Moreover, is there transcriptional convergence of other XPGs (e.g. Tbr1), as the data presented suggest enhancing either XPG is sufficient to restore developmental deficits?

We have identified putative Meis2 targets in PV INs whose expression will be examined.

We will use AAV-DIO-Meis2 with PV-Cre Ribotag mice to boost Meis2 expression in CA3/CA2 PV INs to identify targets of Meis2 in PV INs. We will capture translated mRNAs PV INs with elevated Meis2 levels.

Analysis of transcriptional convergence between XPGs (Meis2 vs Tbr1) is beyond the scope of this study.

10. Meis2 overexpression in Cntnap2+/+ mice appears to worsen performance on the OLM task (Fig. 4c) yet the authors do not comment on this. Similarly, Meis2 overexpression exacerbates performance in social recognition (Fig. 4f), and the authors provide an insufficient explanation ("when GABAergic inhibition is intact increasing MEIS2 dosage does not enhance cognition" [lines 260-261]) for why Meis2 enhancement exacerbates performance on the OLM and social discrimination task. What do the authors mean by intact GABAergic inhibition? Cntnap2+/+ mice have statistically indistinguishable CA2 PN sIPSC amplitude and CA2 PN mIPSC frequency and amplitude from Cntnap2-/- mice. Furthermore, Meis2 enhancement in Cntnap2+/+ mice caused 1 out of the 6 mice to seize (Extended Figure 8a-c); if GABAergic inhibition is indeed intact, what precipitated this seizure?

Line 259: Unlike in *Cntnap2*$^{-/-}$ mice, elevating *Meis2* levels in CA3/CA2 PV INs of *Cntnap2*$^{+/+}$ mice impaired social and spatial cognition.

Network rearrangement following boosting Meis2 in CA3/CA2 PV INs of WT mice may account for impaired cognitive performance and the rare seizure. Note that for analysis of seizures, ECoG recordings were performed 3-4 months following viral transduction as Cntnap2 KO mice exhibit seizures between 4-6 months of age. Our observations document the impact of a manipulation that has not been done before. Therefore, it informs future studies targeting PV INs with transcription factors in WT and NDD risk backgrounds.

11. The authors should describe why Cal-Light is being conducted (over other calcium sensors) and the technical modifications implemented to test PV activation in their model. What is the transfection efficiency of the three employed viruses, and how might poor transfection affect the interpretation put forth? Moreover, the authors provide no representative images of the number of active cells in control/Meis2-boosted Cntnap2+/+ and Cntnap2-/- mice during the reactivation phase. The image of cFOS+/GFP+ cells (Fig. 4h) is not nearly sufficient.

We spent 5 months optimizing Cal-Light in our own lab and worked closely with a collaborator who developed the technique. We will provide all supporting data in addition to new data directly addressing your concerns.

12. It is not specified why the authors chose to test the effects of boosting Meis2 levels on seizure generation other than

the simple fact that Cntnap2-/- mice exhibit spontaneous seizures. According to the SFARI database, MEIS2 mutations are not associated with epilepsy, thus the relevance of this experiment to cognitive restoration needs to be provided.

We are sorry that our reasoning behind selection of Cntnap2 mutant mice was unclear. We chose Meis2 to convey proof of concept for our XPGs as regulators of PV IN plasticity and to demonstrate that restoring CA3/CA2 PV IN plasticity is sufficient to improve cognition and reduces seizures in NDDs.

We chose the Cntnap2 KO model because it exhibits a plethora of developmental deficits and not just a reduction in PV INs and PV IN dysfunction. As such, this model allows us to test the significance and generalizability of targeting experience-dependent PV IN plasticity as a pro-cognitive intervention for NDDs that reduces seizures.

In our manuscript, we convey this rationale twice and we will do a better job conveying it even more clearly in the revised manuscript.

Line 137: Since many NDDs including ASD are characterized by a plethora of developmental deficits, intellectual disability, seizures and impaired social cognition, we selected *Cntnap2* KO (*Cntnap2*$^{-/-}$) mice to test our hypothesis. Specifically, *Cntnap2*$^{-/-}$mice exhibit an array of developmental deficits including loss of hippocampal and cortical PV INs, social and spatial cognitive impairments and seizures by around 4-6 months of age[40-43].

Line 322: To test the hypothesis that loss of experience-dependent PV IN plasticity in CA3/CA2 is a substrate for cognitive impairments and seizures in NDDs, we chose the *Cntnap2* KO model of NDD risk. This was not to model a specific NDD, but because it exhibits a plethora of developmental brain-wide deficits including neuronal migration, myelination, neuronal excitability, loss of GABAergic inhibition and PV INs, cognitive impairments and seizures[40-43].

13. The authors claim that overexpressing Meis2 in PV+ neurons in the DG of Cntnap2-/- mice is sufficient to suppress seizures. However, the data presented are unconvincing. It is surprising that overexpressing Meis2 in an extremely sparse cell population is able to abate seizure generation as measured via ECoG. Moreover, a single control treated Cntnap2-/- mouse appears to be driving this effect (Fig. 5c). To confirm the veracity of this finding, the authors should increase the sample size in Fig. 5 and utilize an acute seizure model (e.g. PTZ, kainic acid, etc.) to determine if Meis2 overexpression suppresses seizures.

We are increasing the sample size for ECoG.
Utilizing an acute seizure model is desirable but beyond the scope of this study.

Minor Concerns

1. In the introduction, the authors state they are testing a “prototypical NDD risk model” (line 99). What makes the Cntnap2-/- mouse a prototypical NDD risk model compared to Fmr1-KO, Tsc1+/-, Tsc2+/-, or Mecp2-/-, for example?

I regret this poor word choice. It is not a prototypical model but a NDD risk model.

2. Supplementary Table 1 needs to be organized between significantly upregulated, downregulated, and unchanged genes. Moreover, the authors state “18 of 67 ASD genes encode histone methyltransferase, histone demethylases, ATP-dependent chromatin remolding factors, transcription factors and coactivators” (lines 114-116), yet these genes are not highlighted in Supplementary Table 1. In its current state, it is unclear which genes are upregulated, downregulated, or unchanged or are associated with a particular functon(e.g. histone demethylation).

Will do.

3. “Significantly upregulated genes included 67 Category 1 ASD SFARI genes” (lines 110-111), however, in Figure 1c, only 47 genes are displayed as Category 1 ASD SFARI genes. Why were these 47 genes presented, and what are the other 20 Category 1 ASD SFARI genes? If select genes were chosen to be presented, why were these genes decided upon?

We will fix this.

4. The following change should be made to lines 171-174, as 1) the current phrasing is ambiguous as to which measure

(frequency or amplitude) changes on PV interneurons, 2) the incorrect Figures are referenced in the text, and 3) Meis2 augmentation does not affect PV IN mEPSC frequency: "Ex vivo whole cell patch-clamp recordings from CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current frequency and amplitude in CA2 PNs compared to Cntnap2+/+ mice, and boosting Meis2 in PV INs reduced sEPSC frequency and amplitude in Cntnap2-/- and Cntnap2+/+ mice (Fig. 3a, Extended Data Fig. 3a)" (lines 171-174).

Sorry that this sentence was mis-interpreted. We will rephrase for greater clarity.

5. The labels for the x-axis in Figure 4 should be present throughout the entire Figure (e.g. Fig. 4c has no x-axis, but the x-axis labels are present in Fig. 4e).
Will fix.

6. Please make sure all abbreviations used in the figures are defined.

Will do.

Referee #2 (Remarks to the Author):

Summary of the key results: This study uncovered experience-dependent plasticity genes (XPGs) that are essential for PV-IN function and are part of genetic risk factors in the context of adjustment of inhibition. Shih et al. found Meis2 as a key XPG and studied the impact of Meis2 expression in restoring hippocampal activity. Using viral vectors, electrophysiology and behavioural assessments, the authors reveal that restoring PV-IN plasticity in the adult hippocampus is sufficient to reverse developmental deficits in a mouse model of autism.

Originality and significance: Whilst this study is of significance and brings substantial insights into neuronal plasticity in the adult brain, the general concept of targeting activity-dependent molecules and manipulating PV-INs to reverse developmental impairments is not new.

Selimbeyoglu A, Kim CK, Inoue M, Lee SY, Hong ASO, Kauvar I, Ramakrishnan C, Fenno LE, Davidson TJ, Wright M, Deisseroth K. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Sci Transl Med. 2017 Aug 2;9(401):eaah6733. doi: 10.1126/scitranslmed.aah6733. PMID: 28768803; PMCID: PMC5723386.

Yap EL, Pettit NL, Davis CP, Nagy MA, Harmin DA, Golden E, Dagliyan O, Lin C, Rudolph S, Sharma N, Griffith EC, Harvey CD, Greenberg ME. Bidirectional perisomatic inhibitory plasticity of a Fos neuronal network. Nature. 2021 Feb;590(7844):115-121. doi: 10.1038/s41586-020-3031-0. Epub 2020 Dec 9. PMID: 33299180; PMCID: PMC7864877.

Prior work has relied on optogenetic stimulation of PV INs and not targeting cell-autonomous mechanisms of exp-dependent PV IN plasticity since the identities of cell-autonomous regulators of hippocampal PV IN exp-dep plasticity were not known (until our discovery was preprinted). Your suggestion that the Greenberg lab's paper (that we cite) conveys precedence for our discovery is biased by the possibility that Meis2 in pyramidal neurons is driving the increase in inhibition of pyramidal cells. We understand your concern because the Greenberg lab showed that cfos in pyramidal neurons dictates inhibitory synaptic inputs on the same pyramidal cells. We will address this concern directly as follows:

-We have generated PV-Cre; Cntnap2 KO bigenic mice and are engineering a Cre-ON AAV DIO Meis2 virus to use in combination with these mice to boost Meis2 exclusively in CA3/CA2 PV INs. We will characterize changes and extent of rescue of inhibitory and excitatory synaptic transmission in DG-CA2 of Cntnap2 KO and WT littermates.

-We will use the CA2 pyramidal cell specific Amigo-2 Cre line in combination with Cre-ON AAV-DIO Meis2 virus to characterize the effect of boosting Meis2 in pyramidal cells on inhibition of these cells.

Data & methodology: The major limitation of the study is the use of a viral construct that is not entirely specific to PV-INs (Extended Fig 2a, b). Meis2 is poorly expressed in PVINs, compared to pyramidal cells. Therefore, the impact of Meis2 expression in pyramidal cells on influencing hippocampal circuits cannot be overlooked. To validate the conclusion that

specific Meis2 expression in PV-INs underlies reversal of developmental deficits in the Cntnap2 KO mice, further analysis of the distribution and expression patterns of Meis2, as well as the physiological properties of the pyramidal cells, is necessary.

See above.

Statistics and biological replicates: Some statistics are missing (e.g. Fig.5), or inconsistent (e.g. Fig2d, top (no statistical differences between groups) vs bottom panel (statistical differences between groups). Certain analyses require a higher number of biological replicates (e.g. Extended data fig1 b: n=2; Extended data fig2 b: n=3; Extended data fig7 d: n=3 for social behaviour) to enable more robust statistical evaluation.

We will clearly convey stats to distinguish between mice and cages (SLEAP).

The seizure analysis (Fig. 5) currently appears to lack sufficient strength to support the statement on line 290 regarding the boosting of Meis2 to efficiently suppress seizures in the Cntnap2 KO mice. Only the number of seizures/day changes between conditions. The statistics (Fig 5c) appear to be driven by a single mouse sitting at 20+ seizures a day (which is likely an outlier), compared to fewer than 2 seizures for the remaining KO mice. How does the occurrence of seizures compare to what has been reported in the literature for this model? In addition, statistical analysis for Fig.5d is required (e.g. Chi Square test).

We are increasing the sample size for ECoG, and will compare our data with the one published study and will perform the requested statistical analysis.

The authors did not report any sex differences in the KO mice compared to WT. On lines 853-855, it is mentioned that "if no statistical difference or interaction between sex were observed, then mice were grouped…". This is surprising. How to compare when the total n for some experiments is <5 (e.g. Fig3, in particular for the behaviour (fig 4)? In particular, please check Extended data fig.7d in which there are only n=3 mice for the behavioural assessment. For some reason, the histograms do not show the same n values (i.e. nose-nose, nose to center, nose to rear and total interactions), despite representing data from the same mice.
We will adjust SABV statement accordingly and will clarify when we are referring to cages vs. mice (Extended data fig.7d).

Conclusions:

-The major concern in the present study is the experimental approach, using a virus that is not entirely specific to PV-INs (line 162-163). Extended Data Fig.2 is crucial as it reveals the quality of the viral infection and type of cells that are affected by the infection. Presented results do not support the statement that selective boosting of Meis2 expression in CA3/CA2 PV INs reverses developmental deficits in the hippocampus and associated behaviour. In fact, only 70% of infected cells seem to be PV INs, with 30% likely to be pyramidal cells, which express high levels of Meis2 (cell in the middle in Extended Data Fig.2a (arrowhead should be unfilled), Extended data Fig. 2b & Fig 2c, see Meis2 expression in non-PV cells vs PV cells). The authors report that Meis2 is expressed at negligibly low levels in a subpopulation of PV interneurons. What is the proportion of Meis2-expressing PV and non-PV cells? Do they all have Meis2 protein? Unfortunately, there is no comparative analysis of Meis2 expression in PV-INs vs pyramidal cells (proportion of expressing cells and mean intensity levels). What is the percentage of PV+ cells infected with AAV-S5E2-Meis2-nls tdtomato? It seems that barely 25% are infected according to Extended Data Fig.2a. In that context, data does not support the conclusion that specific Meis2 expression in PV-INs restores deficits in the Cntnap2 KO mice.

We have generated PV-Cre; Cntnap2 KO bigenic mice and are engineering a Cre-ON AAV-DIO-Meis2 virus to use in combination with these mice to boost Meis2 exclusively in CA3/CA2 PV INs. We will characterize changes and extent of rescue of inhibitory and excitatory synaptic transmission in DG-CA2 of Cntnap2 KO and WT littermates.

We will use the CA2 pyramidal cell specific Amigo-2 Cre line in combination with Cre-ON AAV-DIO Meis2 virus to characterize the effect of boosting Meis2 in pyramidal cells on inhibition of these cells.

-No difference in Meis2 levels in PV cells control vs Cntnap2 KO is reported (Fig.2d top &bottom). How can Meis2 support the beneficial effect of the Meis2 viral infection in that context?

This is precisely the point. Figure 2d reflects no difference between control and Cntnap2 KO at baseline (naïve state) but experience-dependent upregulation is impaired in Cntnap2 KOs.

Line 151: Next, we asked whether *Meis2* expression levels in CA3/CA2 PV INs in adult *Cntnap2*$^{-/-}$ mice and *Cntnap2*$^{+/+}$ littermates change in response to social experience. Multiplex fluorescent *in situ* hybridization for *Meis2*, *PV* and *RGS14* in hippocampal sections obtained from mice habituated to a context (social experience naïve) and mice exposed to a social stimulus in the habituated context revealed a social experience-dependent increase in *Meis2* transcripts (*Meis2* intensity per PV IN and proportion of PV INs that express *Meis2*) in *Cntnap2*$^{+/+}$ mice but not in *Cntnap2*$^{-/-}$ mice (**Fig. 2c-d**).

-Pyramidal cells, which express high levels of Meis2, are likely the main cells responding to the boost in Meis2 expression to trigger a cascade of events involving PV-INs. One could argue that the restoration of perisomatic synapses observed in CA2 in Fig.2 e,f could be an effect of the AAV-S5E2-Meis2-nls tdtomato expression in pyramidal cells? Authors should prove the specificity and discuss this important validation point to solidify results.

We have generated PV-Cre; Cntnap2 KO bigenic mice and are engineering a Cre-ON AAV DIO Meis2 virus to use in combination with these mice to boost Meis2 exclusively in CA3/CA2 PV INs. We will characterize changes and extent of rescue of inhibitory and excitatory synaptic transmission in DG-CA2 of Cntnap2 KO and WT littermates.

We will use the CA2 pyramidal cell specific Amigo-2 Cre line in combination with Cre-ON AAV-DIO Meis2 virus to characterize the effect of boosting Meis2 in pyramidal cells on inhibition of these cells.

- The statement on line 191-192: “Boosting Meis2 in PV INs attenuated…” is an overstatement as pyramidal cells are likely to be affected as well. Evidence with physiological and immunological assessments in pyramidal cells in control and Meis2 viral infection in control and Cntnap2 KO mice are necessary (e.g. Fig.3g: DG inputs principally target CA2 pyramidal cells).

Please see response to your previous comment for ongoing experiments.

- The reason why the authors selected Tbr1 over other key genes to study how they influence PV INs function is not well introduced. Is Tbr1 expressed in all PV INs? In pyramidal cells? In fact, the impact of Tbr1 on hippocampal function is less impressive than the ones observed with Meis2. I am not sure that the current data supports the conclusions from line 233. I recommend this section to be strengthened with more compelling results.

The primary motivation behind performing additional evidence for other XPGs is to demonstrate and contrast the effects of boosting different XPGs in PV INs on PV IN properties (synapse number, synapse physiology, synaptic plasticity). The use of Cntnap2+/+ mice is justified for this purpose. The limited effect of boosting Tbr1 in PV INs of in Cntnap2+/+ mice compared to that seen with Meis2 motivated us to focus on Meis2 for the rescue experiments. We hope that our publicly shared dataset and preliminary characterization of other XPGs will facilitate investigation of these XPGs. However, it is simply beyond the scope of this manuscript to test these different XPGs for their pro-cognitive restorative potential as we have done for Meis2.

If the reviewers would like us to remove the data for other XPGs, we are happy to oblige. We are happy to provide this data for broad access to the neuroscience community on the Sahay lab website.

Absence of qualitative differences between the WT and Cntnap2 KO mice (e.g. social interactions (Extended Data Fig. 7d) and epilepsy (Fig.5; Extended Data Fig. 8)) is surprising. Social alterations represent a key feature of the Cntnap2 KO mice (Penagarikano et al 2011 Cell).

Natural group homecage behavior has not been analyzed before and we did not detect a difference in home cage group social interactions with SLEAPai. We were also surprised that Cntnap2 KO mice showed normal group social interactions

in their homecage. To your point, group home cage social behavior is very different from social interaction in a three-chamber task. Additionally, for SLEAPai recordings mice are housed with littermates of the same genotype.

Cntnap2 KO mice develop seizures around 4-6 months of age and we tested 2-3 months old mice in social cognition tasks. Social behavior of mice used for ECoG was not recorded.

Suggested improvements:
-The conclusions of the study would be significantly strengthened by showing that specific Meis2 expression in PV-INs supports restoration of developmental deficits (e.g. behaviour) in other (genetic and/or non-genetic) models of NDDs (e.g. shank3 ko, valproate acid model etc…).

The extensive characterization that we performed in this study is to provide granular insights into how Meis2-dependent restoration of PV IN plasticity affects circuitry, ensemble properties and behavior. Although not requested by reviewers, we are interested in linking Meis2-dependent changes in PV IN plasticity with emergent network properties such as Sharp-Wave Ripples, SWRs, a neural substrate for memory consolidation. Towards this goal, we are completing analysis of how our manipulation of Meis2-dependent changes in PV IN plasticity restores SWRs in Cntnap2 KO mice.

Analysis of other models of NDD risk are beyond the scope of this study. We are happy to provide a limitations paragraph that conveys that we have not tested these models of NDD risk.

- A discussion point regarding potential compensatory mechanisms from other cells that are affected by the Meis2 viral injection (i.e. pyramidal cells) should be added in the manuscript. Considering that Meis2 maintains intrinsic excitability and terminal arborization in neurons (line 136), it is likely that Meis2 expression shapes pyramidal cell activity and morphology. This point must be addressed, as it is essential for the conclusion of the manuscript (i.e. Direct vs indirect regulation of the PV-IN potential). An analysis of the physiological properties of pyramidal cells under the different experimental conditions is required to confirm that they are not affected, and do not directly contribute to the phenotype observed.

Yes, as conveyed previously, experiments are underway.

-The MFF of the PV INs is lower in the KO and restored by Meis2. This could be synaptically-driven by pyramidal cells, as their firing properties are boosted by Meis2 expression (see extended data fig.3g, right top vs bottom) and/or by PV INs receiving more glutamatergic inputs (Fig. 3e). Recordings as in Fig3i under synaptic blockers could confirm that the different physiological properties between the experimental groups are solely due to different intrinsic properties of PV INs following Meis2 addition.

We will perform experiments to isolate the contribution of CA2 inputs onto PV INs as a driver of changes in PV IN intrinsic excitability.

-What are the target genes of Meis2 in PVIN and pyramidal cells? To understand how Meis2 works, it would be beneficial to include some supporting Cut&Run/ Cut&Tag data.

We have identified putative Meis2 targets in PV INs whose expression will be examined.

We will use AAV-DIO-Meis2 with PV-Cre Ribotag mice to boost Meis2 expression in CA3/CA2 PV INs to identify targets of Meis2 in PV INs. We will capture translated mRNAs PV INs with elevated Meis2 levels.

-In the discussion section, the authors mention that Meis2 expression is virtually absent in the MGE, suggesting that hippocampal PV-INs expressing Meis2 are likely derived from another embryonic region. What about the preoptic area which can represent another source of hippocampal PV interneurons?
Good points to add to the discussion.

References: Overall, references were adequately cited. I could not find any report of Meis2 levels for Reference 37.

Please take a look at Supplementary tables (GSE142546_tpm_all-simplified) for Reference 37: Que et al., *Nature communications* **12**, 108 (2021). https://doi.org/10.1038/s41467-020-20328-4. In their RNA sequencing data, Meis2 is mainly expressed in basket cells. Note, Meis2 is expressed at negligibly low levels in a subpopulation of vertical basket cells.

Clarity and context: Whilst the manuscript effectively outlines the contribution of PV-IN plasticity in shaping the adult brain, it is already well established that PV IN dysfunction represents a hallmark of NDDs. This study uncovered key molecules that are modified upon experience and are linked to NDDs. The justification for focusing on Meis2 and Tbr1 rather than other XPGs is weak. Meis2 is sparsely expressed (line 132), compared to other genes detected (Fig1.c). Whilst the authors claim that specific increase in Meis2 in PV IN restore deficits in the Cntnap2 KO mice, the fact that Meis2 is poorly expressed in PV IN (few cells positive and weak expression) compared to other hippocampal cells must be discussed. The authors elaborated on the impact of PV INs on blanket inhibition and selective inhibition of distinct pyramidal cells (line 349-350) as a potential mechanism to remap network dynamics. However, this raises the question as to how boosting Meis2 expression specifically in a subset of PV INs would be enough to reshape the entire hippocampal circuitry and behaviour. Further discussion is required.

We will address these suggestions to highlight our discovery of XPGs, evidence for Meis2 as a XPG in adult hippocampus and the role of experience-dependent PV IN plasticity as a substrate for cognition in NDDs. Ongoing experiments will shed light on how a small number of PV INs can exert a disproportionate effect on hippocampal circuitry and behavior. In another study from our lab that is under review, we demonstrate that dentate granule cells recruitment of PV IN mediated feed-forward inhibition of CA3/CA2 profoundly reshapes hippocampal circuitry and emergent network properties (Chung and Alipio et al, bioRxiv 2025).

Minor comments:
- Line 207-208: How iLTD is different across groups? Any statistics for that?

It is included in the methods, but yes, we will include the stats.

Could it be possible to add a table of the intrinsic properties of patched cells (e.g latency, AP properties…). This will prove informative.

Yes, will do.

- Line 366: may be, not maybe. And 368: regulate, no regulating.
- Line 370: Please briefly clarify in this sentence the overlapping vs distinct effects of Tbr1 and Meis2.
- Why presenting similar data in fig 3h (IPSC LTD, left) and extended data fig 4b (IPSC ltd, middle)? In addition, the figures only show +/+ ctl conditions. It would be informative to see traces from all conditions in fig 3h left.
- Lines 342-343: Is Meis2 also expressed in a subset of cortical PV INs? What is the percentage there?
- Lines 373-374: The idea needs further clarification. What are the possible explanations or mechanisms behind it?
- From line 374, the authors uncovered hundreds of genes that are altered following experience in PV INs. However, deciphering combinatorial “codes” and understanding their functional relevance remains inaccessible, and this limitation should be explicitly stated.

Yes, to all of the above.

Referee #3 (Remarks to the Author):

This manuscript, entitled, “Pro-cognitive restoration of experience-dependent parvalbumin inhibitory neuron plasticity in neurodevelopmental disorders,” by Yu-Tzu Shih and others examines and tests the molecular and circuit plasticity mechanisms underlying experience-dependent plasticity of parvalbumin-expressing interneurons (PV IN). The authors search for transcriptional regulators that are linked to enhanced mossy fiber-PV transmission, a correlate for experienced-dependent PV In plasticity. They identify several candidates and take a closer look at one in particular, Meis2. They use virally-mediated Meis2 upregulation in the background of a neurodevelopmental mouse model (Cntnap2 -\-). With optogenetics and ex-vivo physiology they examine the consequence of Meis2 up-regulation on

transmission, plasticity and intrinsic properties of synapses on CA2 pyramidal neurons. Furthermore, they test the hypothesis that up-regulation of Meis2 can rescue social cognitive deficits in the NDD mouse model.

The work in this manuscript is highly original. The genetic screen is an invaluable contribution to the field, and these findings may potentially have a lasting impact on our understanding of experience-induced plasticity. It is more in the investigation of the mechanism that the experimental strategies and interpretation of the data have potential and serious confounds. However, the authors have discovered something substantial and important, and they demonstrate that this has unquestionable importance for neurodevelopmental disorders. It is more weaknesses, in my opinion in the experiments that they use to fully understand the mechanism that this paper falls short in its current state.

Major concerns:
1)
The authors use the Cntnap 2 -\- mouse model to examine how up-regulation of Meis2 alters synaptic transmission, plasticity and intrinsic properties in hippocampal area CA2. Because of the importance of hippocampal area CA2 in social memory and established link with numerous psychiatric disorders, this makes some sense. The authors focus their attention on the mossy fiber pathway (MFP) input to PV INs in areas CA2/CA3. While this connection is important, I think that the authors need to expand their perspective and consider the other inputs. In addition to the MFP, the Schaeffer collateral inputs from CA3 synapse onto PV INs in area CA2. Furthermore, CA3 SC inputs also synapse onto CA2 Pyramidal cells. To complicate things more, CA2 PCs projects back to both CA3 interneurons and CA3 pyramidal cells (see Boehringer, et al, 2017). (These are just the intra-hippocampal inputs, there are also hypothalamic and cortical synapses). My point here is that the spontaneous EPSCs in CA2 are coming from many different regions, not just the MFS. And likewise, the spontaneous IPSCs are from inhibitory synapses of neurons that are being recruited by numerous excitatory inputs. Care must be taken to not over-interpret the results of figure three. An increase of sEPSC frequency could correspond to hyperexcitability at an input… or an increase in synapse number. If there is an increase in synapse number, which synapse of the many to choose from? Furthermore, and increase in amplitude becomes even tougher to interpret, given the size of the pyramidal cells dendritic arbor and massive attenuation in the apical dendrite. Could in increase in amplitude indicate an increase in proximal synapses? An increase in AMPA receptors at the post-synaptic site? Evoking transmission and performing input/output curves with electrical stimulation before and after blocking GABA receptors for the different inputs would be a straightforward way of determining which dendritic compartment and class of interneurons are being involved.

We will perform the requested electrophysiological experiments.

The situation for miniature EPSCs and IPSCs is simpler, as the feed-forward aspect of the local circuitry is no longer at play. I find it to be really fascinating how there appears to be no difference at all in the mEPSC amplitude, yet a substantial difference for the sEPCS. Could the authors comment on this? It may be helpful to measure the sEPCs in the presence of GABA receptor blockers. The interpretation that the local inhibitory network or feedforward inhibition may be shunting excitation could be examined this way.

We will perform the requested electrophysiological experiments.

The best way to really understand the PV IN – CA2 synapse would be to perform paired recordings and not rely on multi-synaptic methods of excitation. However, given the size of CA2 and the complexity involved in these experiments, I understand why the authors have relied on different methods.

Agreed

The optogenetics in figure 3 and extended data figure 4 needs to be more carefully interpreted. Channelrhodopsin is expressed in the mossy fiber terminals, and synaptic transmission is being evoked by light stimulation. Transmission will occur by a combination of methods: 1) depolarization of mossy fiber axons, resulting in action potentials and vesicle release, and 2) direct depolarization of synaptic terminals. These two methods will have different dynamics and dependance on light intensity. It is not completely sound to interpret paired-pulse ratios with only one intensity of light stimulation. There needs to be an input-output control performed with and without TTX to demonstrate that the light

intensity used is due to action-potentials only. The data that is there might be fine, they authors need to provide the evidence that the experiment was done properly. The pulse is short-ish (1 ms) and the light is over the hilus, but confirming with TTX would be confirmatory.

Agreed, this control experiment can be done and we can include it in Extended data Fig 4. This does not have to be done in all groups, but in controls, i.e., +/+ mice.

The IPSC LTD experiment is very nicely done (panel h). What plasticity mechanism is this? Delta-opioid mediated at the inhibitory terminals onto the CA2 pyramidal cells? This is consistent with what is seen in the PPR results in extended figure 4. I think the authors could comment or explore a little bit more.

Yes, indeed this is Delta-opioid mediated plasticity (Piskorowski & Chevaleyre, 2013 Journal of Neuroscience) and is consistent with their PPR findings. We are happy to comment.

The whole-cell recording of the PV INs in area CA2 are beautiful. Very nice work. Why not analyze the shape of the action potential? If resting membrane potential was not changed, what about membrane resistance? AHP?

Thank you again! Yes, these measures will be included.

2) The behavior seems very cursory to me.
There is a lot of evidence indicating that novel object location is unchanged when hippocampal area CA2 is lesions, or when inhibitory synaptic plasticity is altered. Are the authors now interested in the DG-CA3 circuit??

Because we target CA3/CA2 PV INs in the stratum lucidum both CA2 and CA3 neurons are affected. That is why we observe a rescue of spatial and social cognition.

Also, in panel a, the differences in viral expression and localization are striking and put into question the results.
We will clearly convey that S5E2-dTom-P2A-nlsdTom (control) results in dTom distributed throughout the neuron whereas S5E2-Meis2-P2A-nlsdTom is localized to the soma. This is why the expression appears very different.

Why is the discrimination index not shown for the social memory testing? This is commonly measured and plotted. Why not have habituation as well? Why only 5 minutes between tests? The time interval chosen is pushing the limit of working memory/ hippocampal-dependent memory. At least 10 minutes would be better… or an hour. This seems very minimal for such an important demonstration of a rescue of a neurodevelopmental mouse model during adulthood.

We did do habituation (Figure 4b). The time interval we used was sufficient for control mice to perform the task and reveal impairments in Cntnap2 KOs.

3) I do not like at all the use of the Cal-Light system without the proper demonstration that the dynamic calcium levels of CA2 pyramidal cells during social exploration are relevant for this system. Where is the negative control? Where is the positive control? There is no prior evidence that social information is encoded in area CA2, more ventral CA1. The immunostaining in panel h is very saturated, and there is no indication of what cell types are labeled (RGS14 or PV staining).
We will provide all supporting data in addition to new data directly addressing your concerns.

To me, the epilepsy experiments look very promising but are also a bit preliminary. Why not look at seizure threshold, or length with stimulation (trans-cornial or septal stimulation?). Pharmacological stimulation? I could probably be convinced if there are good arguments. Furthermore, for the Meis2 animals, why not examine epilepsy-related histological markers?

We are increasing the sample size for ECoG recordings. We will examine epilepsy-related histological markers.

Minor problems:
1) In figure 2C, The images are too saturated and zoomed in to be useful in assessing location in the hippocampal region, or the overall phenomenon that the authors are trying to illustrate. From what I can tell, it appears that Meis2 is not expressed in PV cells, but rather, in CA2 pyramidal cells? Considering that the Meis2 gene was identified following

isolation from PV-Cre; Rpl22HA.HA mice. Could the authors comment on this?
2) The cartoons in Figure 3 (all panels) absolutely must be corrected!! The MFS does not synapse onto distal apical dendrites of CA2!

Will do.

Point-by-point response to reviewers' comments

[redacted]

We thank the editor and the reviewers for their time, constructive critiques, and insightful comments. Over the last year, we have experimentally addressed all 3 reviewers' concerns to ground our conclusions on firm footing. Notably:

1. We developed a genetic-temporal strategy (Adult PV Cre;Cntnap2 KO mice with AAV-DIO-Meis2 injection into CA2/CA3) to express *Meis2* only in adult CA3/CA2 PV INs and assess the impact on excitatory and inhibitory synaptic transmission and cognition. We found that *Meis2* expression in adult CA3/CA2 PV INs is sufficient to restore excitatory and inhibitory synaptic transmission (**new Fig.4**) and cognition (**new Fig.5**) in *Cntnap2* KO mice. These results corroborate our findings obtained using the S5E2 enhancer-based viral strategy to target *Meis2* expression in CA3/CA3 PV INs. We include both PV-Cre targeted *Meis2* expression and S5E2-targeted *Meis2* expression strategies in the revised manuscript.

2. We conducted essential control experiments to address possibility that S5E2-targeting of *Meis2* expression in putative CA2 pyramidal neurons (or non-PV INs) may recruit PV IN mediated inhibition onto CA2 PNs. We provide an in-depth analyses of the S5E2 enhancer-based viral strategy by systematically characterizing target cell populations expressing *Meis2* with immunohistochemistry and electrophysiology. We demonstrate that S5E2-targeted expression of ChR2 does not enable optically evoked excitatory responses in CA2 (**new Extended Fig.1e, new Extended Data Fig.2, new Extended Data Fig.3**). Using CA2 specific Cre mice (Amigo-2 Cre) in combination with AAV-DIO-*Meis*2 we show that *Meis2* expression in CA2 pyramidal neurons does not increase PV IN synapses onto CA2 pyramidal neurons (**new Fig.1e-g**). These results rule out the contribution of *Meis2* expression in CA2 pyramidal cells in mediating inhibition onto CA2 pyramidal cells.

3. Using a mossy fiber electrical stimulation protocol in combination with pharmacology (Gabazine), we independently corroborated a role for increased *Meis2* expression in S5E2-targeted PV INs in enhancing feed-forward inhibition upon CA2 PNs in *Cntnap2*$^{+/+}$ and *Cntnap2*$^{-/-}$ mice and feedforward inhibitory control of excitation upon CA2 PNs in *Cntnap2*$^{-/-}$ mice (**new Extended Data Fig. 2**).

4. We have increased the numbers of mice in both control and experimental groups, performed additional experiments and statistical analyses to establish the seizure-suppressive effects of S5E2-targeted *Meis2* expression in adult CA2/CA2 PV INs of *Cntnap2*$^{-/-}$ mice (**new Fig.6, new Extended Data Fig.9**).

5. We performed LFP recordings in CA1 of *Cntnap2*$^{-/-}$ mice and showed that *Meis2* expression in CA3/CA2 PV INs restores rate, peak amplitude, power and duration of sharp-wave ripples (**new Extended Data Fig.8**).

6. We identified putative targets of MEIS2 in adult CA3/CA2 PV INs (**new Extended Data Fig.10**).

7. We built a fully searchable, curated and annotated XPG portal for unrestricted access by the global neuroscience community (https://xpg-atlas.onrender.com/). Through this portal, researchers can search for their candidate genes on the XPG list and learn about how different XPGs encode high confidence disease risk genes for ASD, BD, SCZ and epilepsy.

All together, we believe the revised manuscript has been greatly improved through peer-review, and we hope the new experiments (consistent with our roadmap provided to the editor) will satisfy reviewers' concerns. We urge all 3 reviewers to look at responses to each reviewer's comments to appreciate how we have provided experimental support to defend the conclusions of this study. Below, we provide our point-by-point responses to each reviewer's comments.

Referee #1 (Remarks to the Author):

The authors identify Meis2 as a novel transcriptional regulator of experience-dependent parvalbumin (PV) interneuron (IN) plasticity. Using the Cntnap2-/- mouse to model a neurodevelopmental disorder, the authors show that Meis2 overexpression remodels excitatory and inhibitory circuitry and E/I imbalance in the DG to CA3/CA2 pathway. This structural remodeling accompanies improvements in performance on a spatial and social cognitive behavioral task.

1. Meis2 is a Category 1 SFARI ASD risk gene, and, although rare missense mutations in MEIS2 are associated with autistic behavior, the relevance of Meis2 to other neurodevelopmental disorders (NDDs)- and Cntnap2-/- mice- is unclear. For example, Meis2 levels in PV-expressing (PV+) cells at baseline and the relative frequency of Meis2+/PV+ neurons are unaltered between Cntnap2+/+ and Cntnap2-/- mice (Fig. 4d). The link between Meis2 dysregulation and Cntnap2 dysregulation is unclear.

We chose the *Cntnap2* KO model because it exhibits a plethora of developmental and cognitive deficits including a reduction in PV INs and PV IN dysfunction. As such, this model allows us to test the significance of targeting experience-

dependent PV IN plasticity (through *Meis2* expression in adult CA3/CA2 PV INs) as a generalizable pro-cognitive, anti-epileptic intervention for NDDs.
In our manuscript, we convey this rationale twice:
Line 156: Since many NDDs including ASD are characterized by a plethora of developmental deficits, intellectual disability, seizures and impaired social cognition, we selected *Cntnap2 KO* (*Cntnap2*$^{-/-}$) mice to test our hypothesis. Specifically, *Cntnap2*$^{-/-}$mice exhibit an array of developmental deficits including loss of hippocampal and cortical PV INs, social and spatial cognitive impairments and seizures by around 6 months of age[41-44].
Line 402: To test the hypothesis that loss of experience-dependent PV IN plasticity in CA3/CA2 is a substrate for cognitive impairments and seizures in NDDs, we chose the *Cntnap2* KO model of NDD risk. This was not to model a specific NDD, but because it exhibits a plethora of developmental brain-wide deficits including neuronal migration, myelination, neuronal excitability, loss of GABAergic inhibition and PV INs, cognitive impairments and seizures[41-44].

2. The authors state that viral upregulation of Tbr1, Bcl11a, Meis2, and Herc1 increases "PV IN synapses in CA2 and CA3 pyramidal neurons (Figure. 1d, Extended Data Fig. 1e)" (lines 130-131). However, the authors present PV puncta, which do not represent bona fide synapses. As a minimum, the authors must present enhanced colocalization of synaptotagmin-2 (syt2) and gephyrin to make this assertion. This claim is repeated for Fig. 2a-b and Fig. 2e-f, and colocalization of Syt2 and gephyrin is necessary to make the claim that Meis2 overexpression rescues CA3/CA2 PV IN synapses. Moreover, it is recommended that Fig. 3b and 3d be moved to Figure 2 to support the notion that Meis2 overexpression rescues CA3/CA2 PV IN synapse number.
We have added Syt2+Gephy+ immunostaining in **new Fig.1** and **new Fig.2.** In addition, by combining immunohistochemistry and electrophysiology (**Fig.3, new Fig.4**, **new Extended Data Fig. 2, new Extended Data Fig. 3**), we unequivocally establish a role for *Meis2* in CA3/CA2 PV INs in mediating inhibition of CA2 pyramidal neurons.

3. It is unclear why the effect of viral upregulation of Herc1 is included in a separate graph in Extended Data Fig. 1e and not included in Fig.1d. If the authors are attempting to state that viral upregulation of all four candidate genes increases PV synapses onto CA2/CA3 neurons, then the data should be presented in the same graph.
We have combined all four candidate XPGs in new **Extended Data Fig. 1**.

4. The authors mention that "Meis2 is expressed at negligibly low levels in a subpopulation of hippocampal PV INs in the hippocampus" (lines 131-132). What PV IN population is expressing Meis2? Your current model overexpresses Meis2 under the S5E2 enhancer element which promotes expression of the construct in basket and chandelier PV interneurons. Is overexpression achieved in basket and chandelier CA2/CA3 PV cells, as targeted? Can overexpression, either generally or in the small population that expresses Meis2, explain why Meis2 overexpression in Cntnap2+/+ mice perform worse on OLM and social recognition compared to their control-treated counterparts?
a. Please take a look at Supplementary tables (GSE142546_tpm_all-simplified) for Reference 37: Que et al., *Nature communications* **12**, 108 (2021). https://doi.org/10.1038/s41467-020-20328-4. Their RNA sequencing data shows that *Meis2* is expressed at negligibly low levels in a subpopulation of vertical basket cells.
b. Both the viral (S5E2-targeted *Meis2* expression) and genetic-temporal strategies (PV-Cre; *Cntnap2* KO +AAV DIO *Meis2*) do not permit distinction between basket cells and chandelier cells, let alone different subtypes of basket cells.
c. In the revised manuscript, at the behest of reviewer 3 we calculated discrimination indexes that are more sensitive to changes in behavior of individual animals. Analysis of discrimination indexes for behavioral readouts suggests that behavior may be altered/different but not significantly impaired (**Fig. 5c, Fig.5f, Fig.5g**). It is possible that network changes and rearrangement in connectivity during the two weeks following targeted *Meis2* expression in adult CA3/CA2 PV INs of WT mice changes cognitive performance and results in the rare seizure **(Extended Data Fig. 9a-b, d)**. MEIS2 regulates a suite of targets (**new Extended Data Fig.10**) including epigenetic regulators, ion channel modulators, cell surface proteins and secreted factors. The overproduction of these proteins in PV INs in a wild-type genetic background may disrupt the dynamic range of circuit functions in unpredictable ways. These changes stand in contrast to the restorative effects seen in a mutant background where the dynamic range of circuitry may be restored, and which is the focus of this study.

5. The authors demonstrate enhanced transcription of Meis2 following social experience in Figure 2c-d. However, Meis2 protein levels must be assessed as protein is the functional readout.

We piloted 5 different commercially available MEIS2 antibodies and after validating one antibody (**new Extended Data Fig.1**) we show that social experience increases MEIS2 protein levels in CA3/CA2 PV INs in **new Fig.2f.**

6. In Extended Data Fig. 2, validation of AAV-S5E2-Meis2-P2A-nlsdTomato and AAV-S5E2-dTom-P2A-nlsdTomato is performed, demonstrating ~70% transfection efficiency in PV+ neurons. What is the transfection efficiency of the other XPG-overexpressing viruses (Tbr1, Bcl11a, and Herc1)? Moreover, the authors should also present histological evidence of Meis2, Tbr1, Bcl11, and Herc1 overexpression in PV+ neurons in Fig. 1d.
We have performed immunostaining for these XPGs (S5E2-targeted expression of Meis2/Tbr1/Bcl11a) in CA3/CA2 PV INs) in **new Extended Data Fig. 1f**. We do not expect the targeting efficiencies for these different XPGs to differ when using the S5E2 enhancer. We have had no luck with Herc1 antibodies. We focused on *Meis2* for both S5E2-targeted and PV-Cre targeted strategies and have provided detailed immunohistochemical and electrophysiological characterization for the S5E2 enhancer-targeted cells.

7. The authors state that "Ex vivo whole cell patch-clamp recordings from PV INs and CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current in CA2 PNs compared to Cntnap2+/+ mice… (Fig. 3a, Extended Data Fig. 3a)" (lines 171-173), yet this reviewer can find no inclusion of sEPSC recordings from PV INs.
We have recorded sEPSCs onto PV INs (**New Fig. 3g**).

8. The author's claim that their analysis of "Meis2 and Tbr1 suggests that these two XPGs independently and differentially contribute to experience-dependent PV IN plasticity" (lines 236-237); however, the authors provide incomplete evidence for this case. 1) No histological evidence is provided to confirm Tbr1 was indeed overexpressed. 2) Tbr1 overexpression was performed in only Cntnap2+/+ mice, and it is fundamentally important to determine if Tbr1 overexpression rectifies synaptic properties in Cntnap2-/- mice, as Meis2 overexpression produced differential responses in Cntnap2+/+ and Cntnap2-/- mice. 3) Does Tbr1 overexpression alter PV s/mEPSC frequency and amplitude?
a. TBR1 immunostaining to quantify the increase in TBR1 protein levels in adult CA3/CA2 PV INs is furnished in **new Extended Data Fig. 1f**.
b. The primary motivation behind providing additional evidence for other XPGs is to demonstrate and contrast the effects of boosting different XPGs in PV INs on PV IN properties (synapse number, synapse physiology, synaptic plasticity). The use of *Cntnap2*$^{+/+}$ mice is justified for this purpose. The limited effects of *Tbr1* expression in PV INs of *Cntnap2*$^{+/+}$ mice compared to that seen with targeted *Meis2* expression is what motivated us to focus on *Meis2* primarily for the rescue experiments and to test our central hypothesis. We hope that our publicly shared XPG dataset and preliminary characterization of other XPGs will facilitate investigation of these XPGs. However, it is simply beyond the scope of this study to test these different XPGs for their pro-cognitive restorative potential in *Cntnap2* KO mice as we have done for *Meis2* in the revised manuscript.

9. Meis2 overexpression in Cntnap2-/- mice does not restore PV IN mEPSC frequency but is sufficient to rescue PV IN mEPSC amplitude (Fig. 3e) and PV IN AP spiking (Fig. 3i). As Meis2 is a transcription factor, it will be helpful to know which of its target genes are transcriptionally enhanced. Moreover, is there transcriptional convergence of other XPGs (e.g. Tbr1), as the data presented suggest enhancing either XPG is sufficient to restore developmental deficits?
We performed ribosomal profiling using AAV-DIO-*Meis2* with PV-Cre;RiboTag mice **in New Extended Data Fig. 10** to identified putative MEIS2 targets in CA3/CA2 PV INs. Analysis of transcriptional convergence between XPGs (*Meis2* vs. *Tbr1*) is beyond the scope of this study. We do not know if targeted expression of *Tbr1* in CA3/CA2 PV INs will rescue the developmental deficits in excitatory and inhibitory synaptic transmission in *Cntnap2* KO mice.

10. Meis2 overexpression in Cntnap2+/+ mice appears to worsen performance on the OLM task (Fig. 4c) yet the authors do not comment on this. Similarly, Meis2 overexpression exacerbates performance in social recognition (Fig. 4f), and the authors provide an insufficient explanation ("when GABAergic inhibition is intact increasing MEIS2 dosage does not enhance cognition" [lines 260-261]) for why Meis2 enhancement exacerbates performance on the OLM and social discrimination task. What do the authors mean by intact GABAergic inhibition? Cntnap2+/+ mice have statistically indistinguishable CA2 PN sIPSC amplitude and CA2 PN mIPSC frequency and amplitude from Cntnap2-/- mice.

Furthermore, Meis2 enhancement in Cntnap2+/+ mice caused 1 out of the 6 mice to seize (Extended Figure 8a-c); if GABAergic inhibition is indeed intact, what precipitated this seizure?

a. Sorry, but we did comment on impaired behavioral performance in OLM task in previous version (Line 259: Unlike in *Cntnap2*$^{-/-}$ mice, elevating *Meis2* levels in CA3/CA2 PV INs of *Cntnap2*$^{+/+}$ mice impaired social and spatial cognition). However, and importantly, at the behest of Reviewer 3 we calculated discrimination indexes that are more sensitive to changes in behavior of individual animals. Analysis of discrimination indexes for behavioral readouts suggests that behavior of *Cntnap2*$^{+/+}$ mice (with targeted expression of *Meis2* to CA3/CA2 PV INs) may be altered/different (see raw data for interaction times) but is not significantly impaired (**Fig. 5c, Fig.5f, Fig.5g, also see ensemble reactivation dataset Fig.5k**).

b. It is possible that network changes and rearrangement in connectivity during the two weeks following targeted *Meis2* expression in adult CA3/CA2 PV INs of *Cntnap2*$^{+/+}$ mice *affects* cognitive performance (see raw data for interaction times) and results in the rare seizure **(Extended Data Fig. 9a-b, d)**. Note that for analysis of seizures, ECoG recordings were performed 3-4 months (even longer timeline for changes in network activity) following viral transduction as *Cntnap2* KO mice exhibit seizures between 6 months of age. MEIS2 regulates a suite of targets (**new Extended Data Fig.10**) including epigenetic regulators, ion channel modulators, cell surface proteins and secreted factors. The overproduction of these proteins in PV INs in a wild-type genetic background may disrupt the dynamic range of circuit functions in unpredictable ways. These changes stand in contrast to the restorative effects seen in a mutant background where the dynamic range of circuitry may be restored. Our observations document the impact of a manipulation that has not been done before. Therefore, it informs future studies targeting PV INs with transcription factors in WT and NDD risk backgrounds.

11. The authors should describe why Cal-Light is being conducted (over other calcium sensors) and the technical modifications implemented to test PV activation in their model. What is the transfection efficiency of the three employed viruses, and how might poor transfection affect the interpretation put forth? Moreover, the authors provide no representative images of the number of active cells in control/Meis2-boosted Cntnap2+/+ and Cntnap2-/- mice during the reactivation phase. The image of cFOS+/GFP+ cells (Fig. 4h) is not nearly sufficient.

We spent 5 months optimizing Cal-Light in our own lab and worked closely with a collaborator/co-author who developed the technique. The transfection efficiency is monitored with one of the Cal-Light viruses expressing tdTomato (AAV-M13-TEV-C-P2A-tdTom) independent of calcium influx or light activation. We have included a new data set with representative images from all groups (**new Extended Data Fig.7e-g**). We did not detect PV INs tagged with GFP (as part of the ensemble) owing to technical limitations or PV IN calcium dynamics (**Extended Data Fig. 7h**).

12. It is not specified why the authors chose to test the effects of boosting Meis2 levels on seizure generation other than the simple fact that Cntnap2-/- mice exhibit spontaneous seizures. According to the SFARI database, MEIS2 mutations are not associated with epilepsy, thus the relevance of this experiment to cognitive restoration needs to be provided.

a. We are sorry that our reasoning behind selection of *Meis2* was unclear. We did not choose *Meis2* to model its role in the human condition (as determined by genotype and associated phenotypes) but rather as a candidate XPG based on its discovery in our XPG screen. We also considered *Tbr1* as an initial candidate XPG but based on its more limited and circumscribed effects on PV IN functions ascertained in WT mice we proceeded with *Meis2* to test our central hypothesis, namely experience-dependent PV IN plasticity is a convergent mechanism for NDD risk genes that can be re-instated in adulthood to reverse developmental deficits in circuitry, network excitability and cognition. In other words, we chose *Meis2* to convey proof of concept for our XPGs as regulators of experience-dependent PV IN plasticity and to demonstrate that restoring CA3/CA2 PV IN plasticity (through *Meis2* expression in adult CA3/CA2 PV INs) is sufficient to reverse developmental deficits in excitation-inhibition, cognition and reduces seizures in NDDs.

b. We chose the Cntnap2 KO model not to model the *Cntnap2*-associated human condition but because it exhibits a plethora of developmental deficits including a reduction in PV INs and PV IN dysfunction, circuit-, network- and cognitive impairments. As such, this model allows us to test the significance and generalizability of targeting experience-dependent PV IN plasticity as a pro-cognitive intervention for NDDs that reduces seizures.

Line 156: Since many NDDs including ASD are characterized by a plethora of developmental deficits, intellectual disability, seizures and impaired social cognition, we selected Cntnap2 KO (*Cntnap2*$^{-/-}$) mice to test our hypothesis. Specifically, *Cntnap2*$^{-/-}$mice exhibit an array of developmental deficits including loss of hippocampal and cortical PV INs, social and spatial cognitive impairments and seizures by around 6 months of age[41-44].

Line 402: To test the hypothesis that loss of experience-dependent PV IN plasticity in CA3/CA2 is a substrate for cognitive impairments and seizures in NDDs, we chose the *Cntnap2* KO model of NDD risk. This was not to model a specific NDD, but because it exhibits a plethora of developmental brain-wide deficits including neuronal migration, myelination, neuronal excitability, loss of GABAergic inhibition and PV INs, cognitive impairments and seizures[41-44]. We characterized MEIS2 as a transcriptional regulator of experience-dependent PV IN plasticity in CA3/CA2 and demonstrated using both genetic and viral strategies that *Meis2*-dependent restoration of experience-dependent PV IN plasticity in CA3/CA2 in adult *Cntnap2* KO mice is sufficient to reverse developmental deficits in excitation-inhibition balance, cognitive impairments and seizure risk.

13. The authors claim that overexpressing Meis2 in PV+ neurons in the DG of Cntnap2-/- mice is sufficient to suppress seizures. However, the data presented are unconvincing. It is surprising that overexpressing Meis2 in an extremely sparse cell population is able to abate seizure generation as measured via ECoG. Moreover, a single control treated Cntnap2-/- mouse appears to be driving this effect (Fig. 5c). To confirm the veracity of this finding, the authors should increase the sample size in Fig. 5 and utilize an acute seizure model (e.g. PTZ, kainic acid, etc.) to determine if Meis2 overexpression suppresses seizures.

a. Sorry to correct you but we are targeting CA3/CA2 PV INs and not DG PV INs.

b.We have increased sample size, performed additional experiments for *Cntnap2*$^{-/-}$mice treated with control AAV (**n= 14 mice**) and AAV-*Meis2* (**n = 12 mice**) and statistical analysis of proportional differences between AAV Ctrl and AAV *Meis2* treated cohorts as requested by the reviewer (Chi Square)(**new Fig.6**). Consistent with our previous result, we find that S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$ mice that have seizures. In addition, we added a statistical comparison of AAV Ctrl and vs. AAV *Meis2* treated *Cntnap2*$^{-/-}$ mice excluding the mouse that has 20+ seizures/day as a new panel in the **new Extended Data Fig.9c**. Exclusion of this mouse from the analysis does not affect any of our findings i.e S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$mice that have seizures. Additionally, we found no difference in proportion of PV INs infected by Control or *Meis2* expressing AAVs in *Cntnap2* KO mice (**new Extended Figure 9d**).

c. In further support of how a small number of PV INs expressing *Meis2* exerts disproportionate effects on hippocampal network activity, we found that S5E2-targeted *Meis2* expression in adult CA3/CA2 PV INs of *Cntnap2*$^{-/-}$mice restores CA1 SWR rate, peak amplitude, ripple power and duration. The concordance of results obtained using S5E2-targeted and PV-Cre targeted approaches strongly supports a role for *Meis2* in CA3/CA2 PV INs in regulating excitatory and inhibitory synaptic transmission, network excitability and cognition (**Fig.3**, **new Fig.4, new Fig. 5 and supporting new extended data**). Lastly, it is notable to mention that in prior work, we showed that enhancing mossy fiber drive onto CA3/CA2 PV INs in *Dyrk1a* $^{+/-}$ was sufficient to restore PV IN intrinsic excitability, the balance between excitatory and inhibitory synaptic transmission onto CA2 and cognition (Shih, Alipio, Sahay, Neuron 2023).

d. We hope you will agree that the inclusion of an additional seizure model in this extensively revised manuscript is beyond the scope of this study.

Minor Concerns

1. In the introduction, the authors state they are testing a “prototypical NDD risk model” (line 99). What makes the Cntnap2-/- mouse a prototypical NDD risk model compared to Fmr1-KO, Tsc1+/-, Tsc2+/-, or Mecp2-/-, for example?

We regret this poor word choice. It is not a prototypical model but a widely used NDD risk model.

2. Supplementary Table 1 needs to be organized between significantly upregulated, downregulated, and unchanged genes. Moreover, the authors state “18 of 67 ASD genes encode histone methyltransferase, histone demethylases, ATP-dependent chromatin remolding factors, transcription factors and coactivators” (lines 114-116), yet these genes are not highlighted in Supplementary Table 1. In its current state, it is unclear which genes are upregulated, downregulated, or unchanged or are associated with a particular functon(e.g. histone demethylation).

We have updated our list (based on updated SFARI database) and highlighted the upregulated and downregulated genes in **new Supplementary Table 1**. We also built a fully searchable, curated and annotated XPG portal for unrestricted access by the global neuroscience community (https://xpg-atlas.onrender.com/).

3. “Significantly upregulated genes included 67 Category 1 ASD SFARI genes” (lines 110-111), however, in Figure 1c, only 47 genes are displayed as Category 1 ASD SFARI genes. Why were these 47 genes presented, and what are the other 20 Category 1 ASD SFARI genes? If select genes were chosen to be presented, why were these genes decided upon?

Please see above comment and visit our XPG portal https://xpg-atlas.onrender.com/. Feedback is welcome.

4. The following change should be made to lines 171-174, as 1) the current phrasing is ambiguous as to which measure (frequency or amplitude) changes on PV interneurons, 2) the incorrect Figures are referenced in the text, and 3) Meis2 augmentation does not affect PV IN mEPSC frequency: "Ex vivo whole cell patch-clamp recordings from CA2 pyramidal neurons (PN) revealed that Cntnap2-/- mice exhibit increased spontaneous excitatory postsynaptic current frequency and amplitude in CA2 PNs compared to Cntnap2+/+ mice, and boosting Meis2 in PV INs reduced sEPSC frequency and amplitude in Cntnap2-/- and Cntnap2+/+ mice (Fig. 3a, Extended Data Fig. 3a)" (lines 171-174).
We have made these edits and corrected references to figures (Lines 189-192).
5. The labels for the x-axis in Figure 4 should be present throughout the entire Figure (e.g. Fig. 4c has no x-axis, but the x-axis labels are present in Fig. 4e).
Done
6. Please make sure all abbreviations used in the figures are defined.
Done

Referee #2 (Remarks to the Author):

Summary of the key results: This study uncovered experience-dependent plasticity genes (XPGs) that are essential for PV-IN function and are part of genetic risk factors in the context of adjustment of inhibition. Shih et al. found Meis2 as a key XPG and studied the impact of Meis2 expression in restoring hippocampal activity. Using viral vectors, electrophysiology and behavioural assessments, the authors reveal that restoring PV-IN plasticity in the adult hippocampus is sufficient to reverse developmental deficits in a mouse model of autism.
Originality and significance: Whilst this study is of significance and brings substantial insights into neuronal plasticity in the adult brain, the general concept of targeting activity-dependent molecules and manipulating PV-INs to reverse developmental impairments is not new.
Selimbeyoglu A, Kim CK, Inoue M, Lee SY, Hong ASO, Kauvar I, Ramakrishnan C, Fenno LE, Davidson TJ, Wright M, Deisseroth K. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Sci Transl Med. 2017 Aug 2;9(401):eaah6733. doi: 10.1126/scitranslmed.aah6733. PMID: 28768803; PMCID: PMC5723386.
Yap EL, Pettit NL, Davis CP, Nagy MA, Harmin DA, Golden E, Dagliyan O, Lin C, Rudolph S, Sharma N, Griffith EC, Harvey CD, Greenberg ME. Bidirectional perisomatic inhibitory plasticity of a Fos neuronal network. Nature. 2021 Feb;590(7844):115-121. doi: 10.1038/s41586-020-3031-0. Epub 2020 Dec 9. PMID: 33299180; PMCID: PMC7864877.

Originality and significance: Prior work has relied on optogenetic stimulation of PV INs and not targeting of cell-autonomous mechanisms of exp-dependent PV IN plasticity since the identities of cell-autonomous regulators of adult hippocampal experience-dependent PV IN plasticity (XPGs) were not known (until our discovery was preprinted). To date even as of this resubmission, there is no evidence for a cell-autonomous factor that coordinates experience-dependent changes in PV IN intrinsic properties, structural reorganization of axonal arborizations and perisomatic synapses in CA3/CA2, regulation of feed-forward inhibition of CA3/CA2, and synaptic plasticity or experience-dependent PV IN plasticity in the adult hippocampus. The other important takeaway, as you are perhaps already aware, is that prior screens that have relied on optogenetic or chemogenetic stimulation of PV INs or broad interventions (enriched experience) to identify regulators of PV IN plasticity have not yielded evidence for experience-dependent PV IN plasticity as a convergent mechanism for NDDs as reflected by the number of upregulated high confidence risk genes (mRNAs) captured in our PV IN translatome and the directionality of change (loss of function) in in the human condition. We built a fully searchable, curated and annotated XPG portal for unrestricted access by the global neuroscience community (https://xpg-atlas.onrender.com/). Through this portal, researchers can search for their candidate genes on the XPG list and learn about how different XPGs encode high confidence disease risk genes for ASD, BD, SCZ and epilepsy.

Your suggestion that the Greenberg lab's paper (that we cite and are very familiar with) conveys precedence for our discovery is predicated on the assumption that *Meis2* overexpression in pyramidal neurons is driving the increase in inhibition of pyramidal cells. We understand your concern because the Greenberg lab showed that cfos in pyramidal neurons dictates inhibitory synaptic inputs on the same pyramidal cells. We agree that this major concern that you raise

throughout your review deserved to be addressed head on with new experiments. The following experiments were done over the last year to **unequivocally establish a role for *Meis2* in CA3/CA2 PV INs in mediating inhibition on CA2 pyramidal neurons.**

a. We developed a genetic-temporal strategy (Adult PV Cre;Cntnap2 KO mice with AAV-DIO-Meis2 injection into CA2/CA3) to express *Meis2* only in adult CA3/CA2 PV INs and assess the impact on excitatory and inhibitory synaptic transmission and cognition. We found that *Meis2* expression in adult CA3/CA2 PV INs is sufficient to restore excitatory and inhibitory synaptic transmission (**new Fig.4**) and cognition (**new Fig.5**) in *Cntnap2* KO mice. These results corroborate our findings obtained using the S5E2 enhancer-based viral strategy to target *Meis2* expression in CA3/CA3 PV INs. We include both PV-Cre targeted *Meis2* expression and S5E2-targeted *Meis2* expression strategies in the revised manuscript.

b. We conducted essential control experiments to address possibility that S5E2-targeting of *Meis2* expression in putative CA2 pyramidal neurons (or non-PV INs) may recruit PV IN mediated inhibition onto CA2 PNs. We provide an in-depth analyses of the S5E2 enhancer-based viral strategy by systematically characterizing target cell populations expressing *Meis2* with immunohistochemistry and electrophysiology. We demonstrate that S5E2-targeted expression of ChR2 does not enable optically evoked excitatory responses in CA2 (**new Extended Fig.1e, new Extended Data Fig.2, new Extended Data Fig.3**).

c.Using CA2 specific Cre mice (Amigo-2 Cre) in combination with AAV-DIO-*Meis*2 we show that *Meis2* expression in CA2 pyramidal neurons does not increase PV IN synapses onto CA2 pyramidal neurons (**new Fig.1e-g**). These results rule out the contribution of *Meis2* expression in CA2 pyramidal cells in mediating inhibition onto CA2 pyramidal cells.

d. Using a mossy fiber electrical stimulation protocol in combination with pharmacology (Gabazine), we independently corroborated a role for increased *Meis2* expression in S5E2-targeted PV INs in enhancing feed-forward inhibition upon CA2 PNs in *Cntnap2*$^{+/+}$ and *Cntnap2*$^{-/-}$ mice and feedforward inhibitory control of excitation upon CA2 PNs in *Cntnap2*$^{-/-}$ mice (**new Extended Data Fig. 2**).

Data & methodology: The major limitation of the study is the use of a viral construct that is not entirely specific to PV-INs (Extended Fig 2a, b). Meis2 is poorly expressed in PVINs, compared to pyramidal cells. Therefore, the impact of Meis2 expression in pyramidal cells on influencing hippocampal circuits cannot be overlooked. To validate the conclusion that specific Meis2 expression in PV-INs underlies reversal of developmental deficits in the Cntnap2 KO mice, further analysis of the distribution and expression patterns of Meis2, as well as the physiological properties of the pyramidal cells, is necessary.

As state above, we agreed that your major concern needed to be tackled head on which we went on to do from every angle. Please see responses above. We have addressed all your major concerns with new data. Thank you for providing a reason to solidify the foundation for our main conclusions.

Statistics and biological replicates: Some statistics are missing (e.g. Fig.5), or inconsistent (e.g. Fig2d, top (no statistical differences between groups) vs bottom panel (statistical differences between groups). Certain analyses require a higher number of biological replicates (e.g. Extended data fig1 b: n=2; Extended data fig2 b: n=3; Extended data fig7 d: n=3 for social behaviour) to enable more robust statistical evaluation.

Our apologies. We have clarified the distinction between mice and cages for SLEAP dataset (**Extended Data Fig.6**). We have increased sample sizes (qRT PCR, S5E2-target cell characterization, seizure dataset). All stats are updated and provided in Supplementary Table 3. We have addressed your concerns.

The seizure analysis (Fig. 5) currently appears to lack sufficient strength to support the statement on line 290 regarding the boosting of Meis2 to efficiently suppress seizures in the Cntnap2 KO mice. Only the number of seizures/day changes between conditions. The statistics (Fig 5c) appear to be driven by a single mouse sitting at 20+ seizures a day (which is likely an outlier), compared to fewer than 2 seizures for the remaining KO mice. How does the occurrence of seizures compare to what has been reported in the literature for this model? In addition, statistical analysis for Fig.5d is required (e.g. Chi Square test).

a. We have increased sample size, performed additional experiments for *Cntnap2*$^{-/-}$mice treated with control AAV (**n= 14 mice**) and AAV-*Meis2* (**n = 12 mice**) and statistical analysis of proportional differences between AAV Ctrl and AAV *Meis2* treated cohorts as requested by the reviewer (Chi Square)(**new Fig.6**). Consistent with our previous result, we find that S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$mice that have seizures. In addition, we added a statistical comparison of AAV Ctrl and vs. AAV *Meis2* treated

Cntnap2$^{-/-}$mice excluding the mouse that has 20+ seizures/day as a new panel in the **new Extended Data Fig.9c**. Exclusion of this mouse from the analysis does not affect any of our findings i.e S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$mice that have seizures. Additionally, we found no difference in proportion of PV INs infected by Control or *Meis2* expressing AAVs in *Cntnap2* KO mice (**new Extended Figure 9d**).

b. The seizure phenotype we report here is similar to published studies of Cntnap2$^{-/-}$ mice. In general, young Cntnap2$^{-/-}$ animals do not show generalized seizures but have multiple signs of hyperexcitability and lower seizure threshold. In older mice, however, seizures have been reported in multiple studies. Our report adds to the literature with 24/7 ECoG monitoring of 6-8 month-old animals and provides quantification of seizure frequency, duration and proportion of mice that have seizures. Specifically: 2-5 month-old Cntnap2-KO mice have been reported to have increased seizure susceptibility, as compared to WT mice, including generalized seizures after PTZ challenge (Jang et al., Sci Adv, 2025). Perturbations in hippocampal rhythmogenesis and limited interictal discharges were also documented by Paterno et al. in 3-4 months old Cntnap2-KO mice (Paterno et al., Cell Rep, 2021.). Seizure phenotypes are more severe in older animals. Penagarikano et al. (Cell, 2011.) observed severe behavioral seizures in 9 out of 10 6+ month-old *Cntnap2*-KO mice. In these older mice, the majority of seizures reached Racine Stage 5. Thomas et al. (Sleep. 2017.) and Wang et al. (Mol Autism, 2025.) report barrages of spike-wave discharges in older Cntanap2$^{-/-}$ mice, consistent with generalized seizures. In *Cntnap2*-KO rats, seizure phenotypes begin earlier, with ECoG and behaviorally recorded seizures documented at 1-4 months of age (Thomas et al., Sleep, 2017).

The authors did not report any sex differences in the KO mice compared to WT. On lines 853-855, it is mentioned that "if no statistical difference or interaction between sex were observed, then mice were grouped…". This is surprising. How to compare when the total n for some experiments is <5 (e.g. Fig3, in particular for the behaviour (fig 4)? In particular, please check Extended data fig.7d in which there are only n=3 mice for the behavioural assessment. For some reason, the histograms do not show the same n values (i.e. nose-nose, nose to center, nose to rear and total interactions), despite representing data from the same mice.

We have clarified our SABV statement accordingly and adjusted statistical reporting. We have also added mouse sex numbers in Figure legends.

Your concern re SLEAP dataset "In particular, please check Extended data fig.7d in which there are only n=3 mice for the behavioural assessment. For some reason, the histograms do not show the same n values (i.e. nose-nose, nose to center, nose to rear and total interactions), despite representing data from the same mice" is well founded since we did not clarify whether n=mice or cages. We have now clarified the distinction between mice and cages for SLEAP dataset (**Extended Data Fig.6**).

Conclusions:

-The major concern in the present study is the experimental approach, using a virus that is not entirely specific to PV-INs (line 162-163). Extended Data Fig.2 is crucial as it reveals the quality of the viral infection and type of cells that are affected by the infection. Presented results do not support the statement that selective boosting of Meis2 expression in CA3/CA2 PV INs reverses developmental deficits in the hippocampus and associated behaviour. In fact, only 70% of infected cells seem to be PV INs, with 30% likely to be pyramidal cells, which express high levels of Meis2 (cell in the middle in Extended Data Fig.2a (arrowhead should be unfilled), Extended data Fig. 2b & Fig 2c, see Meis2 expression in non-PV cells vs PV cells). The authors report that Meis2 is expressed at negligibly low levels in a subpopulation of PV interneurons. What is the proportion of Meis2-expressing PV and non-PV cells? Do they all have Meis2 protein? Unfortunately, there is no comparative analysis of Meis2 expression in PV-INs vs pyramidal cells (proportion of expressing cells and mean intensity levels). What is the percentage of PV+ cells infected with AAV-S5E2-Meis2-nls tdtomato? It seems that barely 25% are infected according to Extended Data Fig.2a. In that context, data does not support the conclusion that specific Meis2 expression in PV-INs restores deficits in the Cntnap2 KO mice.

As stated earlier, we agree that this major concern that your raise throughout the review deserved to be addressed head on with new experiments. The following experiments were done over the last year to **unequivocally establish a role for *Meis2* in CA3/CA2 PV INs in mediating inhibition on CA2 pyramidal neurons and reversing the developmental deficits in PV IN intrinsic excitability, excitatory and inhibitory synaptic transmission and cognition.**

a. We developed a genetic-temporal strategy (Adult PV Cre;Cntnap2 KO mice with AAV-DIO-Meis2 injection into CA2/CA3) to express *Meis2* only in adult CA3/CA2 PV INs and assess the impact on excitatory and inhibitory synaptic transmission and cognition. We found that *Meis2* expression in adult CA3/CA2 PV INs is sufficient to restore excitatory and inhibitory synaptic transmission (**new Fig.4**) and cognition (**new Fig.5**) in *Cntnap2* KO mice. These results corroborate our findings obtained using the S5E2 enhancer-based viral strategy to target *Meis2* expression in CA3/CA3 PV INs. We include both PV-Cre targeted *Meis2* expression and S5E2-targeted *Meis2* expression strategies in the revised manuscript.
b. We conducted essential control experiments to address possibility that S5E2-targeting of *Meis2* expression in putative CA2 pyramidal neurons (or non-PV INs) may recruit PV IN mediated inhibition onto CA2 PNs. We provide an in-depth analyses of the S5E2 enhancer-based viral strategy by systematically characterizing target cell populations expressing *Meis2* with immunohistochemistry and electrophysiology. We demonstrate that S5E2-targeted expression of ChR2 does not enable optically evoked excitatory responses in CA2 (**new Extended Fig.1e, new Extended Data Fig.2, new Extended Data Fig.3**).
c. Using CA2 specific Cre mice (Amigo-2 Cre) in combination with AAV DIO *Meis*2 we show that *Meis2* expression in CA2 pyramidal neurons does not increase PV IN synapses onto CA2 pyramidal neurons (**new Fig.1e-g**). These results rule out the contribution of *Meis2* expression in CA2 pyramidal cells in mediating inhibition onto CA2 pyramidal cells.
d. Using a mossy fiber electrical stimulation protocol in combination with pharmacology (Gabazine), we independently corroborated a role for increased *Meis2* expression in S5E2-targeted PV INs in enhancing feed-forward inhibition upon CA2 PNs in *Cntnap2*$^{+/+}$ and *Cntnap2*$^{-/-}$ mice and feedforward inhibitory control of excitation upon CA2 PNs in *Cntnap2*$^{-/-}$ mice (**new Extended Data Fig. 2**).

-No difference in Meis2 levels in PV cells control vs Cntnap2 KO is reported (Fig.2d top &bottom). How can Meis2 support the beneficial effect of the Meis2 viral infection in that context?
This is precisely the point. **Figure 2d** reflects no difference between control and *Cntnap2* KO at baseline (naïve state) but experience-dependent upregulation is impaired in *Cntnap2* KOs.
In the revised manuscript: **Figure 2c-g** reflects no difference between control and *Cntnap2* KO at baseline (naïve state) but experience-dependent upregulation (transcript and protein) is impaired in *Cntnap2* KOs.
Line 169: Next, we asked whether *Meis2* expression levels in CA3/CA2 PV INs in adult *Cntnap2*$^{-/-}$ mice and *Cntnap2*$^{+/+}$ littermates change in response to social experience (**Fig. 2c**). Multiplex fluorescent *in situ* hybridization for *Meis2*, *PV* and *RGS14* (**Fig. 2d-e**) and immunohistochemistry for corresponding protein levels (**Fig. 2f-g**) in hippocampal sections obtained from mice habituated to a context (social experience naïve) and mice exposed to a social stimulus in the habituated context revealed a social experience-dependent increase in *Meis2* transcripts and protein (*Meis2/*MEIS2 intensity per PV IN and proportion of PV INs that express *Meis2/*MEIS2) in *Cntnap2*$^{+/+}$ mice but not in *Cntnap2*$^{-/-}$ mice (**Fig. 2c-g**).

-Pyramidal cells, which express high levels of Meis2, are likely the main cells responding to the boost in Meis2 expression to trigger a cascade of events involving PV-INs. One could argue that the restoration of perisomatic synapses observed in CA2 in Fig.2 e,f could be an effect of the AAV-S5E2-Meis2-nls tdtomato expression in pyramidal cells? Authors should prove the specificity and discuss this important validation point to solidify results.
As detailed in the our prior responses we agree with you. That is why we went all the way to experimentally test your thesis. Our experiments using viral and genetic-temporal strategies unequivocally demonstrate that targeted *Meis2* expression in CA3/CA2 PV INs of adult *Cntnap2*$^{-/-}$ mice reverses the developmental deficits in PV IN intrinsic excitability, excitatory and inhibitory synaptic transmission in DG-CA2 and cognition. Amigo-2 Cre targeted *Meis2* expression in CA2 pyramidal neurons does not increase PV IN synapses onto CA2 neurons. Additionally, we have provided perhaps the most thorough immunohistochemical and electrophysiological characterization to date for the S5E2-targeting strategy which will be useful for anyone considering its use in hippocampal CA3/CA2.

- The statement on line 191-192: “Boosting Meis2 in PV INs attenuated…” is an overstatement as pyramidal cells are likely to be affected as well. Evidence with physiological and immunological assessments in pyramidal cells in control and Meis2 viral infection in control and Cntnap2 KO mice are necessary (e.g. Fig.3g: DG inputs principally target CA2 pyramidal cells).
Please see response to your previous comment for ongoing experiments. Additionally, we performed your requested experiment (4 groups, virus X genotype) in **new Extended Data Fig. 3**

- The reason why the authors selected Tbr1 over other key genes to study how they influence PV INs function is not well introduced. Is Tbr1 expressed in all PV INs? In pyramidal cells? In fact, the impact of Tbr1 on hippocampal function is less impressive than the ones observed with Meis2. I am not sure that the current data supports the conclusions from line 233. I recommend this section to be strengthened with more compelling results.

The primary motivation behind performing additional evidence for other XPGs is to demonstrate and contrast the effects of boosting different XPGs in PV INs on PV IN properties (synapse number, synapse physiology, synaptic plasticity). The use of *Cntnap2*$^{+/+}$ mice is justified for this purpose. In fact, and as you inadvertently allude to, the limited effects of *Tbr1* expression in PV INs of *Cntnap2*$^{+/+}$ mice compared to that seen with targeted *Meis2* expression is what motivated us to focus on *Meis2* primarily for the rescue experiments and test our central hypothesis. We hope that our publicly shared XPG dataset and preliminary characterization of other XPGs will facilitate investigation of these XPGs. However, it is simply beyond the scope of this study to test these different XPGs for their pro-cognitive restorative potential in *Cntnap2* KO mice as we have done for *Meis2* in the revised manuscript.

Absence of qualitative differences between the WT and Cntnap2 KO mice (e.g. social interactions (Extended Data Fig. 7d) and epilepsy (Fig.5; Extended Data Fig. 8)) is surprising. Social alterations represent a key feature of the Cntnap2 KO mice (Penagarikano et al 2011 Cell).
We were also surprised that we did not detect a difference in home cage group social interactions with SLEAPai. To your point, group home cage social behavior is very different from social interaction in a three-chamber task. Additionally, for SLEAPai recordings mice are housed with littermates of the same genotype.

Suggested improvements:
-The conclusions of the study would be significantly strengthened by showing that specific Meis2 expression in PV-INs supports restoration of developmental deficits (e.g. behaviour) in other (genetic and/or non-genetic) models of NDDs (e.g. shank3 ko, valproate acid model etc…).
The extensive characterization that we performed in this study is to provide the first demonstration of how targeted expression of an XPG in adult CA3/CA2 PV INs in a NDD risk model restores experience-dependent PV IN plasticity, ensemble and network properties (SWRs), cognition and reduces seizure burden. Indeed, we are keen to test the generalizability of our thesis in other NDD risk models (ongoing work that will take 3-5 years) but as you can well appreciate, this undertaking is far beyond the scope of this revised manuscript.

That said, based on our genetic screen (directionality of change of XPGs and LOF genotype in humans), it is not lost on us that impaired experience-dependent PV IN plasticity is likely to be a hallmark of NDDs incl. ASD, BD, SCZ. Indeed, and consistent with your suggestion, we showed that enhancing mossy fiber drive onto CA3/CA2 PV INs in *Dyrk1a* $^{+/-}$ was sufficient to restore PV IN intrinsic excitability, the balance between excitatory and inhibitory synaptic transmission onto CA2 and cognition (Shih, Alipio, Sahay, Neuron 2023). Additionally, based on studies using other NDD risk models (*Tsc2*$^{+/-}$ and *Fmr1*$^{-/-}$), we propose in Line 412 that: XPGs are viable candidates for restoring impaired experience-dependent PV IN plasticity and PV homeostatic plasticity in different NDDs that exhibit deficits in these mechanisms[9,57,58].

- A discussion point regarding potential compensatory mechanisms from other cells that are affected by the Meis2 viral injection (i.e. pyramidal cells) should be added in the manuscript. Considering that Meis2 maintains intrinsic excitability and terminal arborization in neurons (line 136), it is likely that Meis2 expression shapes pyramidal cell activity and morphology. This point must be addressed, as it is essential for the conclusion of the manuscript (i.e. Direct vs indirect regulation of the PV-IN potential). An analysis of the physiological properties of pyramidal cells under the different experimental conditions is required to confirm that they are not affected, and do not directly contribute to the phenotype observed.
Please see our prior detailed responses describing extensive experimental efforts that have addressed this major concern.

-The MFF of the PV INs is lower in the KO and restored by Meis2. This could be synaptically-driven by pyramidal cells, as their firing properties are boosted by Meis2 expression (see extended data fig.3g, right top vs bottom) and/or by PV INs

receiving more glutamatergic inputs (Fig. 3e). Recordings as in Fig3i under synaptic blockers could confirm that the different physiological properties between the experimental groups are solely due to different intrinsic properties of PV INs following Meis2 addition.
New experiments as requested suggest excitatory synaptic transmission does not influence PV IN intrinsic properties (**new Extended Data Figure 2n-q**). These data indeed indicate the different physiological properties between groups are due to targeted Meis2 expression in PV INs.

Line 242: Whole-cell recordings of S5E2-targeted PV INs before and after bath application of CNQX and AP5 suggested that excitatory synaptic transmission does not contribute to *Meis2*-dependent restoration of PV IN excitability in *Cntnap2*$^{-/-}$ mice (**new Extended Data Fig. 2n-q**).

-What are the target genes of Meis2 in PVIN and pyramidal cells? To understand how Meis2 works, it would be beneficial to include some supporting Cut&Run/ Cut&Tag data.
To identify putative MEIS2 targets in PV INs that may regulate experience-dependent PV IN plasticity, we performed qPCR analysis of candidate genes using mRNAs biochemically isolated from CA3/CA2 PV INs of adult PV-Cre Ribotag mice overexpressing *Meis2* in CA3/CA2 PV INs (**Extended Data Fig. 10a**). Our analysis revealed upregulation of mRNAs encoding for a suite of transcription factors (Bcl11a), ion channel regulators (Kcnab1), synaptic adhesion and signaling proteins (Kirrel3, Cdh2, Dcc, Reln, Tanc1), chromatin remodeler (Chd7), and the PV IN-specific synaptic vesicle protein Syt2 (**Extended Data Fig. 10b**).

-In the discussion section, the authors mention that Meis2 expression is virtually absent in the MGE, suggesting that hippocampal PV-INs expressing Meis2 are likely derived from another embryonic region. What about the preoptic area which can represent another source of hippocampal PV interneurons?
The main (and exciting!) point is that experience-dependent plasticity mechanisms mediated by XPGs like *Meis2* do not reflect the developmental origins of cell types but rather flexible use of factors to modify neuronal properties independent of cell identity. This helps understand how *Meis2* modifies properties of both excitatory (sensory neurons) and inhibitory neurons (PV INs, potentially DLS GABAergic projection neurons (Goode et al Neuron 2026).

References: Overall, references were adequately cited. I could not find any report of Meis2 levels for Reference 37.
Please take a look at Supplementary tables (GSE142546_tpm_all-simplified) for Reference 37: Que et al., *Nature communications* **12**, 108 (2021). https://doi.org/10.1038/s41467-020-20328-4. In their RNA sequencing data, *Meis2* is mainly expressed at negligibly low levels in a subpopulation of vertical basket cells.

Clarity and context: Whilst the manuscript effectively outlines the contribution of PV-IN plasticity in shaping the adult brain, it is already well established that PV IN dysfunction represents a hallmark of NDDs. This study uncovered key molecules that are modified upon experience and are linked to NDDs. The justification for focusing on Meis2 and Tbr1 rather than other XPGs is weak. Meis2 is sparsely expressed (line 132), compared to other genes detected (Fig1.c). Whilst the authors claim that specific increase in Meis2 in PV IN restore deficits in the Cntnap2 KO mice, the fact that Meis2 is poorly expressed in PV IN (few cells positive and weak expression) compared to other hippocampal cells must be discussed. The authors elaborated on the impact of PV INs on blanket inhibition and selective inhibition of distinct pyramidal cells (line 349-350) as a potential mechanism to remap network dynamics. However, this raises the question as to how boosting Meis2 expression specifically in a subset of PV INs would be enough to reshape the entire hippocampal circuitry and behaviour. Further discussion is required.

Meis2 is expressed in pyramidal cells (https://hipposeq.janelia.org/full/t1/5279A14E-14AB-11F1-BDB5-17C99DC49958/) and at extremely low levels in PV INs (as conveyed earlier) but is induced by experience in PV INs (this study). We have addressed many of your other points in earlier responses. We will re-emphasize that while "PV IN dysfunction represents a hallmark of NDDs" is widely recognized, evidence linking NDD risk genes directly to experience-dependent PV IN plasticity in the hippocampus is scarce. The directionality of change of XPGs in our screen combined with the fact that many of these XPGs harbor LOF mutations in humans provides the most compelling evidence to date linking distinct properties of PV INs directly with these candidate XPG NDD risk genes. This is the hypothesis that we tested in this study.

On the subject of how a small number of PV INs exert disproportionate effects on hippocampal network activity:
a. With regards to how a small number of PV INs expressing *Meis2* exerts disproportionate effects on hippocampal network activity, we found that S5E2-targeted *Meis2* expression in adult CA3/CA2 PV INs of *Cntnap2*$^{-/-}$ mice restores CA1 SWR rate, peak amplitude, power and duration (**new Extended Data Fig 8**).
b. Several studies from our lab have demonstrated that mossy fiber inputs onto PV INs (trigger for experience-dependent PV IN plasticity, the focus of this study) exert profound changes in circuitry and network properties in CA1 (SWRs). Specifically, we showed that enhancing mossy fiber drive onto CA3/CA2 PV INs in *Dyrk1a* $^{+/-}$ was sufficient to restore PV IN intrinsic excitability, the balance between excitatory and inhibitory synaptic transmission onto CA2 and cognition (Shih, Alipio, Sahay, Neuron 2023). Second, in a study that is under revision we show that adult-born dentate granule neurons recruit PV IN mediated inhibition to enhance duration of SWRs (CA1) (Chung and Alipio et al, 10.21203/rs.3.rs-6087158/v1). Third, in a prior study, we causally linked PV IN mediated feed-forward inhibition in DG-CA3/CA2 with SWR-spindle coupling in CA1-ACC and CA1 ensemble stability and specificity (hippocampal-prefrontal networks)(Twarkowski et al 2022 *eLife* https://elifesciences.org/articles/70586) Fourth, chemogenetic inhibition of CA1 PV INs impairs SWR-spindle coupling (CA1-ACC) (Xia et al *eLife* 2017 https://elifesciences.org/articles/27868).

Minor comments:
- Line 207-208: How iLTD is different across groups? Any statistics for that?
Occurrence of iLTD is included in the statistics (Supplementary Table 3). Only percentage of occurrence within group is compared.
Could it be possible to add a table of the intrinsic properties of patched cells (e.g latency, AP properties…). This will prove informative.
We now provide additional intrinsic properties (**new Extended Data Fig.2n-q and new Extended Data Fig.3c-e**).
- Line 366: may be, not maybe. And 368: regulate, no regulating. Corrected
- Line 370: Please briefly clarify in this sentence the overlapping vs distinct effects of Tbr1 and Meis2. Done
- Why presenting similar data in fig 3h (IPSC LTD, left) and extended data fig 4b (IPSC ltd, middle)? In addition, the figures only show +/+ ctl conditions. It would be informative to see traces from all conditions in fig 3h left.
The IPSC LTD and EPSC LTP are Yes and No comparisons for all groups. The graphs will look the same for each group therefore a single representative graph was used to depict the LTD/LTP.
- Lines 342-343: Is Meis2 also expressed in a subset of cortical PV INs? What is the percentage there? Yes, 5-HT$_{3A}$R expressing cortical interneuron subtypes. https://www.nature.com/articles/ncomms14219
- Lines 373-374: The idea needs further clarification. What are the possible explanations or mechanisms behind it?
- From line 374, the authors uncovered hundreds of genes that are altered following experience in PV INs. However, deciphering combinatorial "codes" and understanding their functional relevance remains inaccessible, and this limitation should be explicitly stated.
Yes, to all of the above.
Thank you for your detailed critique of our manuscript.

Referee #3 (Remarks to the Author):

This manuscript, entitled, "Pro-cognitive restoration of experience-dependent parvalbumin inhibitory neuron plasticity in neurodevelopmental disorders," by Yu-Tzu Shih and others examines and tests the molecular and circuit plasticity mechanisms underlying experience-dependent plasticity of parvalbumin-expressing interneurons (PV IN). The authors search for transcriptional regulators that are linked to enhanced mossy fiber-PV transmission, a correlate for experienced-dependent PV In plasticity. They identify several candidates and take a closer look at one in particular, Meis2. They use virally-mediated Meis2 upregulation in the background of a neurodevelopmental mouse model (Cntnap2 -\-). With optogenetics and ex-vivo physiology they examine the consequence of Meis2 up-regulation on transmission, plasticity and intrinsic properties of synapses on CA2 pyramidal neurons. Furthermore, they test the hypothesis that up-regulation of Meis2 can rescue social cognitive deficits in the NDD mouse model.

The work in this manuscript is highly original. The genetic screen is an invaluable contribution to the field, and these findings may potentially have a lasting impact on our understanding of experience-induced plasticity. It is more in the investigation of the mechanism that the experimental strategies and interpretation of the data have potential and

serious confounds. However, the authors have discovered something substantial and important, and they demonstrate that this has unquestionable importance for neurodevelopmental disorders. It is more weaknesses, in my opinion in the experiments that they use to fully understand the mechanism that this paper falls short in its current state.
We appreciate the strong support from Reviewer 3. And we had added new experiments to address Reviewer 3 concerns.

Major concerns:
1)The authors use the Cntnap 2 -\- mouse model to examine how up-regulation of Meis2 alters synaptic transmission, plasticity and intrinsic properties in hippocampal area CA2. Because of the importance of hippocampal area CA2 in social memory and established link with numerous psychiatric disorders, this makes some sense. The authors focus their attention on the mossy fiber pathway (MFP) input to PV INs in areas CA2/CA3. While this connection is important, I think that the authors need to expand their perspective and consider the other inputs. In addition to the MFP, the Schaeffer collateral inputs from CA3 synapse onto PV INs in area CA2. Furthermore, CA3 SC inputs also synapse onto CA2 Pyramidal cells. To complicate things more, CA2 PCs projects back to both CA3 interneurons and CA3 pyramidal cells (see Boehringer, et al, 2017). (These are just the intra-hippocampal inputs, there are also hypothalamic and cortical synapses). My point here is that the spontaneous EPSCs in CA2 are coming from many different regions, not just the MFS. And likewise, the spontaneous IPSCs are from inhibitory synapses of neurons that are being recruited by numerous excitatory inputs. Care must be taken to not over-interpret the results of figure three. An increase of sEPSC frequency could correspond to hyperexcitability at an input… or an increase in synapse number. If there is an increase in synapse number, which synapse of the many to choose from? Furthermore, and increase in amplitude becomes even tougher to interpret, given the size of the pyramidal cells dendritic arbor and massive attenuation in the apical dendrite. Could in increase in amplitude indicate an increase in proximal synapses? An increase in AMPA receptors at the post-synaptic site? Evoking transmission and performing input/output curves with electrical stimulation before and after blocking GABA receptors for the different inputs would be a straightforward way of determining which dendritic compartment and class of interneurons are being involved.
We have expanded our study to include Schaeffer collateral or mossy fiber driven excitation and inhibition input-output curves recorded from CA2 pyramidal neurons (**new Extended data figure 2a-j**). We also assess feedforward inhibition in these specific pathways and pharmacologically validate pathway specific stimulation.

The situation for miniature EPSCs and IPSCs is simpler, as the feed-forward aspect of the local circuitry is no longer at play. I find it to be really fascinating how there appears to be no difference at all in the mEPSC amplitude, yet a substantial difference for the sEPCS. Could the authors comment on this? It may be helpful to measure the sEPCs in the presence of GABA receptor blockers. The interpretation that the local inhibitory network or feedforward inhibition may be shunting excitation could be examined this way.
We have assessed pathway specific feedforward inhibition (**new Extended Data Fig.2**). We applied GABA receptor blockers (Gabazine) before and after assessing EPSC/IPSC input output curves (**new Extended Data Fig.2**). As predicted, *Cntnap2* KO mice exhibit impaired feedforward inhibition in both mossy fiber DG-CA2 and Schaffer collateral CA3-CA2 pathways. S5E2-targeted *Meis2* expression in the KO mice restored this inhibition.

The best way to really understand the PV IN – CA2 synapse would be to perform paired recordings and not rely on multi-synaptic methods of excitation. However, given the size of CA2 and the complexity involved in these experiments, I understand why the authors have relied on different methods.
Agree. Thank you for your understanding.

The optogenetics in figure 3 and extended data figure 4 needs to be more carefully interpreted. Channelrhodopsin is expressed in the mossy fiber terminals, and synaptic transmission is being evoked by light stimulation. Transmission will occur by a combination of methods: 1) depolarization of mossy fiber axons, resulting in action potentials and vesicle release, and 2) direct depolarization of synaptic terminals. These two methods will have different dynamics and dependance on light intensity. It is not completely sound to interpret paired-pulse ratios with only one intensity of light stimulation. There needs to be an input-output control performed with and without TTX to demonstrate that the light intensity used is due to action-potentials only. The data that is there might be fine, they authors need to provide the

evidence that the experiment was done properly. The pulse is short-ish (1 ms) and the light is over the hilus, but confirming with TTX would be confirmatory.
We now provide pharmacological validation of our pathway specific optic stimulation (**new Figure 4l**) and pathway specific electrical stimulation (**new Extended Data Figure 2e, j**).

The IPSC LTD experiment is very nicely done (panel h). What plasticity mechanism is this? Delta-opioid mediated at the inhibitory terminals onto the CA2 pyramidal cells? This is consistent with what is seen in the PPR results in extended figure 4. I think the authors could comment or explore a little bit more.
Yes, indeed this is Delta-opioid mediated plasticity (Piskorowski & Chevaleyre, 2013 Journal of Neuroscience) and is consistent with their PPR findings.

The whole-cell recording of the PV INs in area CA2 are beautiful. Very nice work. Why not analyze the shape of the action potential? If resting membrane potential was not changed, what about membrane resistance? AHP?
Thank you. We now provide additional intrinsic properties (**new Extended Data Fig. 2n-q** and **new Extended Data Fig. 3c-e**).

2) The behavior seems very cursory to me.
There is a lot of evidence indicating that novel object location is unchanged when hippocampal area CA2 is lesions, or when inhibitory synaptic plasticity is altered. Are the authors now interested in the DG-CA3 circuit??
Because we target CA3/CA2 PV INs both CA2 and CA3 neurons are affected. That is why we observe a rescue of spatial and social cognition. Additionally, as we show, targeted *Meis2* expression in CA3/CA2 PV INs of *Cntnap2*$^{-/-}$mice restores (CA1) SWR rate, peak amplitude, ripple power and duration (**new Extended data Fig. 8**). Lastly, the idea that CA2 solely contributes to social information processing is incorrect. First, and as you also point out, CA3 and CA2 exert reciprocal inhibition onto each other. Second, a recent study using the Amigo-2 Cre to selectively silence CA2 pyramidal neurons found impairments in spatial cognition 10.1007/s12264-025-01535-9 . Third, both context and social stimuli trigger remapping in CA2 (Alexander 2016 Nat Comm https://www.nature.com/articles/ncomms10300).

Also, in panel a, the differences in viral expression and localization are striking and put into question the results.
Yes, the expression of dTom is different for these two viruses. This is because the control AAV has two dTom cassettes and the second dTom cassette is preceded by a nls(nucleus location signal). With this control virus dTom distributes throughout the neuron: neurites and nucleus. In contrast, in the *Meis2* expressing virus the first dTom was replaced with *Meis2* leaving the second dTom preceded by a nls intact. Therefore, dTom distributes only in the nucleus.

In the methods, we now convey that that S5E2-dTom-P2A-nlsdTom (control) results in dTom distributed throughout the neuron whereas S5E2-Meis2-P2A-nlsdTom is localized to the nucleus.

Why is the discrimination index not shown for the social memory testing? This is commonly measured and plotted. Why not have habituation as well? Why only 5 minutes between tests? The time interval chosen is pushing the limit of working memory/ hippocampal-dependent memory. At least 10 minutes would be better… or an hour. This seems very minimal for such an important demonstration of a rescue of a neurodevelopmental mouse model during adulthood.
We calculated discrimination indexes that are more sensitive to changes in behavior of individual animals (**Fig.5f, Fig.5h**). The behavior schedule is conveyed in **new Fig.5d** and new **extended Figure 5a**. It includes habituation to arena and chamber. We determined the interval as part of validating sensitivity/dynamic range of task i.e. WT littermates can perform the task while *Cntnap2* KO mice exhibited impaired performance.
Note that analysis of discrimination indexes for behavioral readouts suggests that behavior of *Cntnap2*$^{+/+}$ mice (with targeted expression of *Meis2* to CA3/CA2 PV INs) may be altered/different (see raw data for interaction times) but is not significantly impaired (**Fig. 5c, Fig.5f, Fig.5g, also see ensemble reactivation dataset Fig.5k**).

3) I do not like at all the use of the Cal-Light system without the proper demonstration that the dynamic calcium levels of CA2 pyramidal cells during social exploration are relevant for this system. Where is the negative control? Where is the positive control? There is no prior evidence that social information is encoded in area CA2, more ventral CA1. The immunostaining in panel h is very saturated, and there is no indication of what cell types are labeled (RGS14 or PV

staining).
We spent 5 months optimizing Cal-Light in our own lab and worked closely with a collaborator/coauthor who developed the technique. We have included a new experimental dataset to include the negative control, context vs. social for Cal-Light, and new representative images for all groups (**new Extended Figure 7a-g**). We also included immunostaining for PV+ cells in CA2/CA3 (**new Extended Figure 7h**). We did not detect PV INs tagged with GFP (as part of the ensemble) owing to technical limitations or PV IN calcium dynamics (**Extended Data Fig. 7h**).

To me, the epilepsy experiments look very promising but are also a bit preliminary. Why not look at seizure threshold, or length with stimulation (trans-cornial or septal stimulation?). Pharmacological stimulation? I could probably be convinced if there are good arguments. Furthermore, for the Meis2 animals, why not examine epilepsy-related histological markers?
We have increased sample size, performed additional experiments for *Cntnap2*$^{-/-}$mice treated with control AAV (**n= 14 mice**) and AAV-*Meis2* (**n = 12 mice**) and statistical analysis of proportional differences between AAV Ctrl and AAV *Meis2* treated cohorts as requested by the reviewer (Chi Square)(**new Fig.6**). Consistent with our previous result, we find that S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$ mice that have seizures. In addition, we added a statistical comparison of AAV Ctrl and vs. AAV *Meis2* treated *Cntnap2*$^{-/-}$ mice excluding the mouse that has 20+ seizures/day as a new panel in the **new Extended Data Fig.9c**. Exclusion of this mouse from the analysis does not affect any of our findings i.e S5E2-targeted *Meis2* expression in CA3/CA2 PV INs significantly reduces seizure frequency and the proportion of *Cntnap2*$^{-/-}$mice that have seizures. Additionally, we found no difference in proportion of PV INs infected by Control or *Meis2* expressing AAVs in *Cntnap2* KO mice (**new Extended Figure 9d**). We have added a general marker for reactive gliosis (GFAP^{+} cell numbers) in new **Extended Figure 9h** but this was not helpful.

Minor problems:
1) In figure 2C, The images are too saturated and zoomed in to be useful in assessing location in the hippocampal region, or the overall phenomenon that the authors are trying to illustrate. From what I can tell, it appears that Meis2 is not expressed in PV cells, but rather, in CA2 pyramidal cells? Considering that the Meis2 gene was identified following isolation from PV-Cre; Rpl22HA.HA mice. Could the authors comment on this?
We have replaced images and added new data for MEIS2 immunohistochemistry (**new Fig.2**). Yes, you are correct, Meis2 is expressed in pyramidal cells. Meis2 expression is very low in CA3/CA2 PV INs but is induced by experience (**Fig. 2**) and increased mossy fiber drive, a trigger of experience-dependent PV IN plasticity (our screen, **Fig. 1**).

2) The cartoons in Figure 3 (all panels) absolutely must be corrected!! The MFS does not synapse onto distal apical dendrites of CA2!
Thank you for pointing this out. These cartoons have been corrected.

[redacted]

Point-by-point response to reviewers' comments

[redacted]

Authors' responses are provided below in blue.

Referee #1 (Remarks to the Author):

This paper is vastly improved, and the authors have addressed many methodological concerns. Some concerns remain:

-Sample sizes remain low in some studies. In most cases, the authors can get away with a low sample size of 4-5, as the effect sizes are large and/or the variance is low. However, there are a few examples when a low sample size is worrisome. For example, the negative data in Figure 1g could be a consequence of under sampling, as the sample size is low and the variability is relatively high compared to other measurements. This lack of effect could easily be a Type II error that very much changes the interpretation of the data (e.g. – suggesting that the Meis2 effect is exclusively driven by its upregulation in PV+ interneurons rather than also through its upregulation in pyramidal neurons).

The quantification of PV-CA2 PN synapses following targeted Meis2 overexpression in Amigo-2+ CA2 PNs was performed **as part of validation of use of S5E2 enhancer to target Meis2 expression in CA3/CA2 PV INs**. The main concern raised by reviewer #2 (and rightly so) was that CA2 PNs *potentially targeted by the S5E2 Meis2 AAV* may mediate the increase in inhibition onto CA2 PNs. We addressed this major, valid concern head on with extensive electrophysiological characterization of PV INs, CA2 PNs and S5E2 targeted cell populations following injection of S5E2-Meis2 AAVs into dorsal CA3/CA2. To the best of our knowledge, this evidence (now a part of the revised manuscript) constitutes the most detailed characterization of the S5E2 enhancer performed to date. To reiterate from our prior Response:

"We conducted essential control experiments to address possibility that S5E2-targeting of *Meis2* expression in putative CA2 pyramidal neurons (or non-PV INs) may recruit PV IN mediated inhibition onto CA2 PNs. We provide an in-depth analyses of the S5E2 enhancer-based viral strategy by systematically characterizing target cell populations expressing *Meis2* with immunohistochemistry and electrophysiology. We demonstrate that S5E2-targeted expression of ChR2 does not enable optically evoked excitatory responses in CA2 (**new Extended Fig.1e, new Extended Data Fig.2, new Extended Data Fig.3**)".

Additionally, we used a CA2 specific Cre mice (Amigo-2 Cre) in combination with AAV-DIO-*Meis*2 to assess changes in PV-CA2 PN synapses following overexpression of Meis2 in Amigo-2+ CA2 PNs. With N=5 animals per group (mice=independent variable) and analysis of 3 sections per animal and 12 images per animal (For CA3, we scored 18 images/mouse), we did not detect a significant increase in PV IN-CA2 PN synapses (**new Fig.1e-g**). **It is important to note that this experiment does not provide direct validation of the S5E2 enhancer's specificity with regards to CA2 PNs, but instead is designed to address a slightly different question: How does selectively targeting Meis2 expression only in CA2 PNs affect PV IN-CA2 PN synapses? This is why we did not pursue any further characterization of this strategy.**

Lastly, and again to reiterate, direct proof for how *Meis2* expression in CA3/CA2 PV INs impacts circuitry comes from our genetic-temporal strategy (Adult PV Cre;Cntnap2 KO mice with AAV-DIO-Meis2 injection into CA2/CA3). Our results using this approach corroborate our findings obtained using the S5E2 enhancer-based viral strategy to target *Meis2* expression in CA3/CA3 PV INs.

-Given the emphasis on the Meis2-driven upregulation of PV interneuron synapses onto CA3 and CA2 pyramidal neurons, --if feasible-- it would be informative to perform triple-labeling studies to include a neuronal marker and/or triple-labeling studies including a dendritic marker to more incisively define sites of PV synaptogenesis. Alternatively, one could conceivably use Tdtomato-labeled PNs instead of neuronal/dendritic markers to examine anatomical targets of enhanced PV synaptogenesis.

The singular most important readout for functional PV IN-CA2 PN synapses comes from electrophysiological analyses, which we have done. With regards to your concern in the first round, we performed Syt2/gephyrin colocalization to label

PV IN-PN synapses. PV IN-PN synapses are perisomatic (PN cell bodies are labeled with RGS14 for CA2 and DAPI (CA3)) and this is clearly conveyed in our images. It is therefore not clear to us how labeling with a neuronal marker will provide any additional information. Now, the dynamics of enhanced PV synaptogenesis in CA3/CA2 is interesting and we are beginning 3D reconstructions of target PNs but alas, this is simply beyond the scope of this study.

The importance of PV interneurons in experience-dependent plasticity, cognition, and seizures is well established. This study adds novelty by showing, in a genetic model with dysregulated Meis2 expression, that rescue of experience-dependent Meis2 upregulation in PV interneurons can reinstate these important functions of parvalbumin interneurons.
Thank you for your insights, comments and time.

Referee #2 (Remarks to the Author):
This manuscript presents a comprehensive and experimentally rigorous investigation into experience-dependent plasticity genes (XPGs) and their role in parvalbumin interneuron (PV-IN) function in the adult hippocampus. The authors identify Meis2 as a key regulator and demonstrate, through a combination of viral targeting strategies, electrophysiology, and behavioural assays, that restoring PV-IN plasticity in adulthood can reverse circuit and behavioural deficits in a Cntnap2 knockout mouse model. That said, as previously noted, the broader conceptual framework of modulating inhibitory circuitry to rescue neurodevelopmental phenotypes is not entirely new. The novelty here lies in the identification of Meis2 and the demonstration of its cell-autonomous role in PV-IN plasticity.

Overall, the revised manuscript has been substantially strengthened. The authors have clearly invested significant effort in addressing my concerns, and the additional experiments provide strong support for their central conclusions. A major strength of the study is the breadth and depth of experimental validation. In particular, the authors show that Meis2 expression in PV-INs is sufficient to restore excitatory/inhibitory balance, hippocampal network activity (including sharp-wave ripples), and behaviour. They implemented a genetic-temporal strategy (PV-Cre; AAV-DIO-Meis2) to restrict expression to adult CA3/CA2 PV interneurons and provided appropriate controls to validate the specificity of the S5E2 enhancer-based viral approach. In addition, seizure analyses have been strengthened through increased sample sizes and improved statistical rigor. The identification of putative downstream targets of MEIS2, together with the XPG portal, represents a valuable resource for the community.
These additions significantly increase confidence that the observed phenotypes are primarily driven by PV-IN-specific mechanisms rather than off-target effects. The study provides important insights into cell-autonomous regulators of experience-dependent PV-IN plasticity. This work advances the field by identifying a molecular regulator linking experience-dependent transcriptional programs to circuit function.

I suggest one minor point that would further improve the discussion. The manuscript highlights that Meis2 is expressed at low baseline levels in PV-INs but is induced by experience. Given its relatively sparse expression compared to pyramidal neurons, it would be useful to more clearly explain how modulation within a subset of PV-INs can produce large-scale network and behavioural effects.
We are currently performing high density single unit recordings to understand how Meis2 expression in PV INs influences PN activity and emergent network properties. It is our hope that these studies (beyond the scope of this manuscript) will begin to inform the precise sequelae of events underlying PV IN-PN interactions as they relate to SWRs.

As conveyed previously:
On the subject of how a small number of PV INs exert disproportionate effects on hippocampal network activity:
a. With regards to how a small number of PV INs expressing *Meis2* exerts disproportionate effects on hippocampal network activity, we found that S5E2-targeted *Meis2* expression in adult CA3/CA2 PV INs of *Cntnap2*$^{-/-}$ mice restores CA1 SWR rate, peak amplitude, power and duration (**new Extended Data Fig 9**).
b. Several studies from our lab have demonstrated that mossy fiber inputs onto PV INs (trigger for experience-dependent PV IN plasticity, the focus of this study) exert profound changes in circuitry and network properties in CA1 (SWRs). Specifically, we showed that enhancing mossy fiber drive onto CA3/CA2 PV INs in *Dyrk1a* $^{+/-}$ was sufficient to restore PV IN intrinsic excitability, the balance between excitatory and inhibitory synaptic transmission onto CA2 and cognition (Shih, Alipio, Sahay, Neuron 2023). Second, in a study that is under revision we show that adult-born dentate granule

neurons recruit PV IN mediated inhibition to enhance duration of SWRs (CA1) (Chung and Alipio et al, 10.21203/rs.3.rs-6087158/v1). Third, in a prior study, we causally linked PV IN mediated feed-forward inhibition in DG-CA3/CA2 with SWR-spindle coupling in CA1-ACC and CA1 ensemble stability and specificity (hippocampal-prefrontal networks)(Twarkowski et al 2022 *eLife* https://elifesciences.org/articles/70586) Fourth, chemogenetic inhibition of CA1 PV INs impairs SWR-spindle coupling (CA1-ACC) (Xia et al *eLife* 2017 https://elifesciences.org/articles/27868).

Conclusion: This is a strong and substantially improved manuscript. I would like to commend the authors for the significant effort invested in addressing the reviewers' concerns and for the thorough additional experiments. Overall, the work provides compelling evidence that Meis2 acts as a cell-autonomous regulator of PV-IN plasticity in the adult hippocampus. I support publication after minor revision addressing the point above.
Thank you for your insights, comments and time.

Referee #3 (Remarks to the Author):
In this exceptional work, the authors use an original and effective screen to identify the homeobox gene Meis221 as a regulator of experience dependent plasticity in areas CA2 and CA3 of the hippocampus. THe authors then demonstrate in a heroic show of force how this gene can be harnessed to rescue fast spiking PV cell properties and corresponding cognitive deficits in neurodevelopmental disorders in adulthood.

I congratulate the authors on a remarkable contribution to the field. All of my comments have been thoughtfully addressed. This manuscript is comprehensive and the new experiments, while extensive, are very well presented and performed. The manuscript is well-written.
Thank you for your insights, comments and time.