nature portfolio

Peer Review File

Chromatin remodeling factor BAF155 coordinates oligodendroglial-neuronal communications linked to regional myelination and autism-like behavioral deficits in mice

Corresponding Author: Professor Jianqin Niu

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Wang et al, present a very interesting paper highlighting the role of BAF155, a chromatin remodeling factor, in the di;erentiation and myelination of oligodendrocyte progenitor cells by integrating the expression of several genes related to OPC-neuronal synaptic regulation. The authors discuss the e;ects of BAF155 KO at the NPC, OPC and OL level with intriguing results. A major concern is how well does BAF155 homozygous KO model heterozygous mutations in the ASD patient population.

1. BAF155 in the CNS regulates OL di;erentiation and myelination
1. In the NPC het, are the number of Olig2+ cells di;erent between genotypes? Is the reduction of mature OLs due to reduced expression of MBP or CC1 at the mature stage or a reduction in the total number of OLs? How about the PDGFRa+ cells. Could it be an issue with the progression of the OPCs into the committed and mature stages or is it an overall reduction in the number of progenitors?
2. Related to Fig 1 and Fig S2
1. Why is there a decrease in PV interneurons? Are the cells dying or are the number of interneuron progenitors low and, hence, fewer cells?
3. IF panels need to be brightened, especially the DAPI channel (e.g. Fig1F, Fig3A). Single panels/channel (preferentially in greyscale to improve contrast) should be added to help with the visualization of the data (eg. Fig 1F, 3F).
4. Are the fluorescence intensities normalized to Dapi counts or Dapi area (as done on Fig 3F quantification)?
5. Figure 3
1. Fig 3A
1. If possible, please include a full brain image (low magnification or stitched)
2. Olig2 staining may be helpful to evaluate if the MBP di;erences are due to OL loss or reduced expression of the protein.
2. Fig 3D and 3E: similar comment. Co-staining with Olig2 may be helpful for the quantification.
3. The lysolecithin-induced demyelination experiments are a bit confusing. How do we know that the injection produced a lesion? A control experiment demonstrating a demyelination lesion was induced is necessary.
6. Figure 2
1. The results section states miniature EPSCs were recorded but the figure and figure legend state spontaneous EPSCs. Which is correct? Based on frequency and amplitudes I am guessing these are sEPSCs and sIPSCs. What is causing the reduced frequency of spontaneous events in pyramidal neurons? Supp Figure 1E,F suggest that axons density and neuron numbers

are not altered. Presumably spontaneous network activity is reduced due to reduced myelination of axons? It would be good to demonstrate this by recording miniature synaptic events, as the TTX would block network activity, presumably mEPSC and mIPSC frequency and amplitude would be unchanged in BAF155 mutants?
2. How many neurons were recorded in each animal? The figure legends states each data point is a mouse.
7. Figure 4
1. For ChIP-seq experiments, how was the antibody validated for its specificity for BAF155? In supp Figure 2, the BAF155 antibody is producing a signal in BAF155fl/fl mouse x PDGFRa-CreER. This could be explained by ine;icient recombination, but could also be due to non-specific BAF155 antibody.
8. Supplemental Figure 1
1. The e;ect of BAF155 heterozygous mutation is quite small, and the majority of the experiments were performed using homozygous KO, which severely limits the relevance of this work in relation to ASD. Patients with BAF155 mutation will only have heterozygous mutations.
9. Supplemental Figure 2
1. In Supp figure 2C and 2D, there is a consistent reduction in CC1+ and MAG+ staining in the right quadrant of the images. What is the exact region of the mPFC that is imaged and is this imaging region consistent between animals?
10. Where all genotypes of mice given tamoxifen?
11. There is a growing body of evidence from several ASD mouse models related to myelination defects in ASD. A discussion of this literature in the introduction and/or discussion should be included.

Minor concerns:
• Abstract, line 54: eliminate the period in the middle of the sentence.
• In the results section describing Figure 3F – instead of “in vivo specific regulatory mechanism”, maybe refer to this as a non-cell autonomous e;ect.
• Figure 5G – a split y-axis may help to display the data points better.
• Y-axis labels are missing space in “fold change”.
• Pg 20, what is meant by “most of these susceptible genes do not reproduce ASD phenotype”.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)

Summary:
In this manuscript from Wang et al, the authors investigate the role of the chromatin remodeler BAF155 in oligodendroglial function. They generate a new conditional loss-of-function mouse line, BAF155fl/fl mice, then cross this line to NestinCreERT2 to hit most brain cells, and PDGFRaCreERT2 to remove BAF155 from OPCs specifically. Analysis of these mice showed reduced numbers of mature oligodendrocytes and myelin production (i.e. hypomyelination) especially in white matter as well as mPFC and hippocampus. At the physiological level, loss of OPC-expressed BAF155 decreased excitatory and inhibitory miniatures onto pyramidal neurons in mPFC and hippocampus. Behaviorally, removing BAF155 from OPCs impairs social behaviors, and increases repetitive and anxiety-like behaviors consistent with symptoms of ASD in humans. When the BAF155fl/fl; PDGFRaCreERT2 mouse line was subjected to a demylination model, OPCs without BAF155 were less capable of differentiating. Chip-seq and DEG analysis of purified committed OPCs revealed gene targets of BAF155 related to synaptic function. They then suggest, based upon structural experiments and Ca2+ imaging, that neuron-OPC synapses are disrupted by loss of BAF155.
Overall, this is a well-written study with high quality, well-organized, and clear figures. I think the topic of how neurons and OPCs communicate in the brain is highly relevant and timely, and the connection with autism increases interest in the work. I think the paper is a particularly good fit for Nature Communications based upon its strength. I only have two main suggestions, as you will see below, but I feel the suggested experiments are really important to address prior to publication, especially providing more extensive evidence that the newly generated mouse line behaves as expected.

Major comments:
1. This appears to be the first reported publication of this new BAF155fl/fl mouse line. However, perhaps I missed it, but I cannot find in the paper the rigorous validation of the line. How do we know that BAF155 is actually disrupted in these mice? Only exon 4 was floxed, so a truncated protein could still exist. To trust in any of these results, a supplemental figure needs to be added validating (1) normal expression of BAF155 in BAF155fl/fl mice versus wildtype mice of the same background

strain when Cre or tamoxifen is absent, and (2) the efficient loss of BAF155 protein from most brain cells in the NestinCreERT2 line and from OPCs specifically in the PDGFRACreERT2 line. These are necessary experiments to interpret the findings of the study.
2. In several places, including lines 354-356, the authors indicate that BAF155 disrupts neuron-OPC synaptic connectivity. To support this conclusion, the authors should assess functional synaptic connectivity between neurons and OPCs using acute slice electrophysiology. In the absence of such an analysis, the claim that BAF155 loss dysregulates neuron-OPC synapses is not fully supported by the data.

Minor comments:
1. A lot of speculation around gene expression results, this should be limited or moved to the discussion.

Reviewer #4

(Remarks to the Author)
The study entitled " Chromatin remodeling factor BAF155 coordinates oligodendroglial-neuronal communications linked to regional myelination and autism-like behavioral deficits " by Wang et al. investigates the role of BAF155 in OPCs in the context of ASD. They use mouse models to delete BAF155 in OPCs in mice to show myelination defects, altered synaptic activity and behaviour, and they correlate these features to altered expression of synaptic genes in OPCs. This is overall very interesting and relevant findings, deserving publication in Nat. comm.
I have several comments that might help to make even better the manuscript.
Major comments:
1.) In the graphical abstract as well as in regard to Figure 6 the authors claimed to have studied chromatin accessibility. This is not correct, since BAF155 ChIP signals could correlate with altered accessibility but do not show it. For a mechanistic explanation of the altered transcription, indeed an ATAC survey or nucleosome positioning of/in the BAF155 deleted OPCs might be of relevance. Correlation to the observed changes in gene expression would be of benefit in addition. Please correct also the first sentence referencing to Fig. 6A for the above mentioned reason, that BAF presence it not necessarily correlated to accessibility changes.
2.) The authors claim that the altered expression of synaptic proteins upon BAF155 deletion leads to impaired OPC-neuron transmission. However, they only show altered Ca-transients in OPCs, or synaptic responses in pyramidal neurons independent from each other. It would be nice to see stimulation of neurons and record altered response in OPCs, which would indeed confirm altered synaptic transmission or communication, as written by the authors. Without this experiments there is the correlation between the events, but not the transmission per se. As the authors identified specific receptors on the OPC side, the relevance of synaptic transmission through these specific receptors can be strengthened by applying selective inhibitors to the WT condition.
3.) How is the connection between altered chromatin in OPCs, myelination defects in axons, altered Ca transients in OPCs, and altered synaptic protein expression in OPCs? Among the target genes, are there as well myelin affecting genes as a missing functional link? How do the authors explain and validate experimentally the different correlations they observe?
4.) On page 11 the authors start the paragraph with " To investigate why loss of BAF155 impairs OPC differentiation in vivo but not in vitro,..." however, the answer to this question is not given by the experimentation. In addition to this, it is not clear whether the ChIP-seq was done on in vivo cells (as suggested by using purified committed progenitors) or on in vitro cultured cells (as suggested by using medium at day 1 and 5). Please clarify - and resolve the difference between native, i.e. in vivo and cultured cells.
5.) " The enrichment analysis of these shared genes such as Ank3 (Ankyrin-3 61), Chl1 (Close Homolog of L1 62), Map1b (Microtubule-associated protein 1b 63), and Mbp 64, indicated their substantial participation in essential biological processes, including neuron projection development, developmental growth, and axon development (Figure 6I)." As the authors claim the substantial participation in essential biological processes, they should integrate these genes in their characterisation to establish the missing functional links between their observations. As a proof of principle that these are indeed essential components linking BAF, neurotransmitter signaling and neuronal development, one of them might be used to establish the mechanistic link between OPC BAF functions, impacting myelination, and neuronal function/development.

Minor comments:
Abstract:
1. Through the shortening of information in the Abstract, some statements become unprecise:
"...BAF155, a newly identified chromatin remodeling factor linked to autism susceptibility, is primarily expressed in committed OPCs." BAF155 is not newly identified per se - it is perhaps novel in its implication in autism. Further, BAF155 is a member of ncBAF and nBAF; it has been shown to be active in cortical progenitors during embryonic development (10.1016/j.isci.2018.05.014). Therefore the claim that BAF155 is primarily expressed in OPCs seems superficial. The authors phrase much better in the introduction - here, it is formulated in a misleading way.
Further, the authors used contribute/contributing in the same sentence and inserted a "." instead of "," in the last sentence.

Results:
2. "...stage (Figure 4G). In addition, an extensive and significant enrichments of..." please correct

3." the reduced number of myelinated axons and increased G-ratios in Baf155 knockout mice at both P14 and 8 weeks of age (Figure 1D)." Please explain G-ratios for a broader audience.

4. The authors observed differences for GABA and Glutamatergic neurons (Fig. 1E, 6G, 6H). Please explain these

differences in regard to potential neuronal functions and correlate to the electrophysiological findings.

5. "neither it affected proliferation OPCs (Figure S2F)." Please correct

6. In regard to Figures 3 and 6 the authors observed differences in mPFC and hippocampus. If the myelination defect is higher in mPFC compared to the hippocampus, but responses on the targets is higher in hippocampus, it seems that the processes are decoupled? Maybe the authors can explain this?

7. The origin of several data sets is not clear and need to be referenced by giving the respective numbers of their deposition to publicly accessible databases:
• RNAseq upon GABA and Glutamate
• Chip-seq H3K27Ac and H3K4me3
• Chip-seq of BAF155
Please give the original data source and make sure these are publicly available to the readership.

8."... which result in regional myelin development and contribute to the diverse onset of symptoms associated with ASD." Do the authors refer to local defects in myelination?

9. Figure legends, Figures:
Please give n numbers for all experiments, even if individual data points are given.
It is a very good way to indicate the p-values in the graphs as done for S1D - why not doing it consistently for all?
Figure 4: 1d and 5d brains is not understandable; please describe what is used as material correctly. Also give n-numbers and statistics for 4I.

Discussion:
Maybe the authors could elaborate on how the communication between OPC receiving inputs from neurons can be related to abnormal neuronal functions in more detail and by citing the relevant literature regarding the transmitters involved?
What are these transmitters, and how do the authors integrate their findings of interneurons being heavily impacted, and OPCs responding differently to GABA or Glutamate?

Methods:
For the demyelinating mouse model, please precise which age and how long the mice were exposed.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have adequately addressed our concerns, except for a few minor edits requested below. It is our opinion the manuscript is suitable for publication.

1. Figure legend for supplementary figure one is missing the word supplementary in the legend title.
2. Concentrations for TTX, BNQX and AP-5 should be in micromolar not millimolar.
3. Never heard of BNQX, I have heard of CNQX and DNQX.
4. Pg. 9, a better explanation for the differing results between sEPSC and mEPSC data would be helpful. It currently states "suggesting that the reduction in spontaneous network activity is attributable to decreased axonal myelination" should also include statements regarding that the no difference in mEPSC frequency and amplitude suggests that synaptic density appears to be intact, and only AP-dependent transmission is affected based on sEPSC data, which indicates mylenation defects may be effection AP-dependent synaptic transmission.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
The authors have addressed my concerns. The paper is now suitable for publication in Nat Comms

Reviewer #4

(Remarks to the Author)
The authors addressed my comments to enhance their manuscript. There is few things left, that could be added and

corrected in the manuscript.
1. Lines 235-236: the authors write about potential enhancer regions. As the H3K27ac data are available and used in the manuscript, I suggest to map the BAF155 binding sites and H3K27ac enrichment and to strengthen the statement, that "enhancers" are bound by BAF155. Presently, without this information, it is more suitable to write about regulatory regions, as intragenic regions are not per se enhancers.
2. Line 260: instead of "specific for H3K27Ac" it would be more suitable to write "enriched for".
3. Line 278: correct "labled"
4. Line 321: correct "play"
5. Line 426: change "triggers" to "triggering"
6. Line 427: change to " in the present study,"
7. Line 430: change "response" to "respond"
8. Line 442: change to "provides an instructional"
9. Line 447: change to "sensing the neuronal"
10. Line 451: change "oligodendroglial lineage" to "oligodendrocytes"
11. Line 460: change to "and humans"

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Wang et al, present a very interesting paper highlighting the role of BAF155, a chromatin remodeling factor, in the differentiation and myelination of oligodendrocyte progenitor cells by integrating the expression of several genes related to OPC-neuronal synaptic regulation. The authors discuss the effects of BAF155 KO at the NPC, OPC and OL level with intriguing results. A major concern is how well does BAF155 homozygous KO model heterozygous mutations in the ASD patient population.

1. BAF155 in the CNS regulates OL differentiation and myelination
In the NPC het, are the number of Olig2+ cells different between genotypes?
Is the reduction of mature OLs due to reduced expression of MBP or CC1 at the mature stage or a reduction in the total number of OLs? How about the PDGFRa+ cells. Could it be an issue with the progression of the OPCs into the committed and mature stages or is it an overall reduction in the number of progenitors?

Response: We sincerely appreciate this comment. To address it, we performed immunostaining experiments to clarify these issues. In addition to OLIG2 staining, we also performed the co-staining for PDGFRα/OLIG2, IP_3R-II/OLIG2 and CC1/OLIG2 in the mPFC of *Nestin*^{CreERT2};*Baf155*$^{fl/+}$ mice. Our results confirmed that the number of OLIG2+ cells was decreased in *Nestin*^{CreERT2};*Baf155*$^{fl/+}$ mice, as well as the numbers of PDGFRα+ OPCs and CC1+ OLs. These results suggest that a reduction of total number of OLs, and an overall decrease in oligodendroglial population. We have added this data in the **New Figure S1F,** and a statement in the limitations of Discussion section that further studies are still needed to elucidate the mechanism underlying the overall reduction of oligodendroglial lineage in *Nestin*^{CreERT2};*Baf155*$^{fl/+}$ mice.

To further determine whether the reduction of total number of OLs due to reduced total number of oligodendroglial lineage or a reduction in the oligodendroglial differentiation, we quantified the proportion of OPCs by measuring the ratio of PDGFRα/OLIG2 co-staining, the proportion of committed OPCs by IP_3R-II/OLIG2 co-staining, and the proportion of mature OL by CC1/OLIG2 co-staining. Both the OPC and OL ratios were decreased in *Nestin*^{CreERT2};*Baf155*$^{fl/+}$ mice, consistent with the decreased OPC an OL numbers. However, the ratio of committed OPCs significantly increased, indicating a block in the transition from committed OPCs to mature OLs. This finding supports our hypothesis that BAF155 is essential for OPC-neuron synaptic connections, which are critical for subsequent differentiation and myelination. We have added this data in Result

section, and highlighted in yellow.

Regarding the question "Is the reduction of mature OLs due to reduced expression of MBP or CC1 at the mature stage or a reduction in the total number of OLs", MBP and CC1 are widely used as the mature markers for OLs; without their expressions, oligodendroglial lineage cells can't be classified as mature OLs. As shown in the **New Figure S1F**, the mature OL ratio decreases while the committed OPC ratio increases, implying again that BAF155-KO impairs the differentiation of OPCs.

2. Related to Fig 1 and Fig S2

Why is there a decrease in PV interneurons? Are the cells dying or are the number of interneuron progenitors low and, hence, fewer cells?

Response: We are sorry for the lack of clarity in our manuscript. In our study, we found a significant decrease of MBP positive area on PV positive axons by MBP/PV co-staining, whereas no difference was found in PV interneuron numbers or SMI32 positive axons. We have revised the description of this part to make it more accurate: "Fluorescence microscopy revealed that the MBP-positive areas on parvalbumin (PV)-positive axons of interneurons, assessed through MBP/PV co-staining, decreased significantly when compared to WT controls. Specifically, there was an 86.86±11.71% reduction at P14 and a 28.68±3.15% reduction at 8 weeks of age **(New Figure 1F, G)**. Loss of BAF155, however, did not affect the SMI32 positive axons **(New Figure 1F, G)**, the numbers of OLIG2-positive and PDGFRα-positive oligodendroglial lineage cells **(Figure S2F, S2G)**, or the proliferation of OPCs **(Figure S2G)**".

3. IF panels need to be brightened, especially the DAPI channel (e.g. Fig1F, Fig3A). Single panels/channel (preferentially in greyscale to improve contrast) should be added to help with the visualization of the data (eg. Fig 1F, 3F).

Response: We thank the reviewer for this comment. Based on the reviewer's suggestion, we have brightened the DAPI channel in **New Figure 1F and 3A**. Additionally, we have replaced single channel with greyscale to enhance the contrast in **New Figure 1F and 3F**.

4. Are the fluorescence intensities normalized to Dapi counts or Dapi area (as done on Fig 3F quantification)?

Response: We thank the reviewer for this comment. We have added this information in the Statistics of Method section and the Figure legend of Fig3F: "For the analysis of representative images of MBP/PDGFRα staining in OPC cultures, the fluorescence

intensities were normalized for the total DAPI nuclear area value". Because the size of DAPI-labelled nuclei is generally consistent, this quantity should represent and correlate with changes in DAPI area (see below reference).

Bolognesi MM, Dall'Olio L, et al. Seeing or believing in hyperplexed spatial proteomics via antibodies: New and old biases for an image-based technology. Biol Imaging. 2024 Oct 23;4:e10. doi: 10.1017/S2633903X24000138. eCollection 2024.

5. Figure 3

1. Fig 3A

If possible, please include a full brain image (low magnification or stitched)

2. Olig2 staining may be helpful to evaluate if the MBP differences are due to OL loss or reduced expression of the protein.

Fig 3D and 3E: similar comment. Co-staining with Olig2 may be helpful for the quantification.

3. The lysolecithin-induced demyelination experiments are a bit confusing. How do we know that the injection produced a lesion? A control experiment demonstrating a demyelination lesion was induced is necessary.

Response: We thank the reviewer for these valuable comments. Based on the reviewer's suggestion, we have added the low magnification images of MBP staining of different regions in our **New Figure S3A, S3E**.

To evaluate if the MBP differences are due to OL loss or reduced expression of myelin protein, we also have added the extra data of CC1/OLIG2/PDGFRα staining of different regions in **New Figure S3B-S3D, S3F-S3H**. These new results indicated a significant reduction in CC1-positive mature OLs, but no differences in OLIG2+ or PDGFRα+ cells, suggesting that the MBP difference of different regions was attributable to the reduction of CC1+ mature OLs rather than the reductions of OLIG2+ or PDGFRα+ cells.

For the Plp^{CreERT};$Baf155^{fl/fl}$ mice, we also have provided the extra data of CC1/OLIG2 co-staining, and MAG in situ hybridization in **New Figure S4C, S4D**, confirming that depleting BAF155 in OLs does not induce obvious defects in oligodendroglial differentiation and myelination.

In addition, for the lysolecithin-induced demyelination experiments, we have included the results of MBP and DAPI staining to define the lesion areas in mice, as shown in **New Figure S4A, S4B**. This model has been widely used in previous studies both in our lab and by other researchers (see below references).

Niu J, Tsai HH, Hoi KK, et al. Aberrant oligodendroglial-vascular interactions disrupt the blood-brain barrier, triggering CNS inflammation. Nat Neurosci. 2019;22(5):709-718.

Niu J, Yu G, Wang X, et al. Oligodendroglial ring finger protein Rnf43 is an essential injury-specific regulator of oligodendrocyte maturation. Neuron. 2021;109(19):3104-3118.e6.

Fancy SP, Harrington EP, et al. Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. Nature Neuroscience. 2011;14(8):1009-16.

Arnett HA, Fancy SP, et al. bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. Science. 2004;306(5704):2111-5.

6. Figure 2

1. The results section states miniature EPSCs were recorded but the figure and figure legend state spontaneous EPSCs. Which is correct? Based on frequency and amplitudes I am guessing these are sEPSCs and sIPSCs. What is causing the reduced frequency of spontaneous events in pyramidal neurons? Supp Figure 1E,F suggest that axons density and neuron numbers are not altered. Presumably spontaneous network activity is reduced due to reduced myelination of axons? It would be good to demonstrate this by recording miniature synaptic events, as the TTX would block network activity, presumably mEPSC and mIPSC frequency and amplitude would be unchanged in BAF155 mutants?
2. How many neurons were recorded in each animal? The figure legends states each data point is a mouse.

Response: We thank the reviewer for his/her careful reading. “Miniature excitatory postsynaptic currents (mEPSC) and miniature inhibitory postsynaptic currents (mIPSC)” shown in the results section should be “spontaneous EPSCs and IPSCs”, which we have corrected. In addition, we performed electrophysiological experiment as suggested. We recorded miniature synaptic currents in the same regions. For isolating mEPSCs, we continuously infused sodium channel blocker tetrodotoxin (TTX, 1 mM) into the ACSF. For recording mIPSCs, we used a special pipette solution containing CsCl and administrate the AMPA receptor antagonist BNQX (10 mM) and NMDA receptor antagonist AP-5 (50 mM) or TTX (1 mM) + BNQX (10 mM) + AP-5 (50 mM) into the ACSF throughout the recording. Our results showed that the frequency and amplitude of mEPSCs and mIPSCs remained unchanged in BAF155 knockout mice **(New Figure 2C, D)**, suggesting that the reduction in spontaneous network activity is attributable to decreased axonal myelination. We have added this new result in our Result and Method sections.

In the figure legend of Fig2A-B, each data point represents a single neuron cell. We are sorry for this mistake in our description. We apologize for this error in our description. This has been corrected in the current version of our manuscript.

7. Figure 4

1. For ChIP-seq experiments, how was the antibody validated for its specificity for BAF155? In supp Figure 2, the BAF155 antibody is producing a signal in BAF155fl/fl mouse x PDGFRa-CreER. This could be explained by inefficient recombination, but could also be due to non-specific BAF155 antibody.

Response: We thank the reviewer for this comment. For ChIP-seq experiments, we selected the antibody from CST (catalog number 11956), which is widely used in ChIP assays and has been validated for specificity (see below references). We also confirmed its specificity for BAF155 by showing the markedly reduced BAF155 staining observed in tissues from BAF155-KO mice. The residual BAF155 signal detected in *PDGFR*α^{CreER}*;BAF155*$^{fl/fl}$ mice is likely attributable to incomplete recombination.

Mondal S, Ramanathan M, Miao W, et al. PROBER identifies proteins associated with programmable sequence-specific DNA in living cells. *Nat Methods*. 2022;19(8):959-968.

Zhang HF, Hughes CS, Li W, et al. Proteomic Screens for Suppressors of Anoikis Identify IL1RAP as a Promising Surface Target in Ewing Sarcoma. *Cancer Discov*. 2021;11(11):2884-2903.

Hu A, Chen G, Bao B, et al. Therapeutic targeting of CNBP phase separation inhibits ribosome biogenesis and neuroblastoma progression via modulating SWI/SNF complex activity. *Clin Transl Med*. 2023;13(4):e1235.

Harlow ML, Chasse MH, Boguslawski EA, et al. Trabectedin Inhibits EWS-FLI1 and Evicts SWI/SNF from Chromatin in a Schedule-dependent Manner. *Clin Cancer Res*. 2019;25(11):3417-3429.

Wu J, Krchma K, Lee HJ, et al. Requisite Chromatin Remodeling for Myeloid and Erythroid Lineage Differentiation from Erythromyeloid Progenitors. *Cell Rep*. 2020;33(7):108395.

8. Supplemental Figure 1

1. The effect of BAF155 heterozygous mutation is quite small, and the majority of the experiments were performed using homozygous KO, which severely limits the relevance of this work in relation to ASD. Patients with BAF155 mutation will only have heterozygous mutations.

Response: We thank the reviewer for raising an important point about the translational relevance of our work. We acknowledge that the heterozygous BAF155 mutation produces a modest yet statistically significant effect, whereas the most of our mechanistic results were from homozygous knockout mice. Most ASD-associated genes (such as CHD8, PTEN, see below references) exhibit only mild or subclinical neurodevelopmental alterations when heterozygously deleted. For instance, CHD8$^{+/-}$ mice display modest

brain overgrowth and subtle behavioral changes; whereas PTEN$^{+/-}$ mice show white-matter abnormalities with relatively limited behavioral deficits. These examples illustrate that heterozygous mutations typically produce fine-scale cellular or molecular perturbations rather than dramatic phenotypes. In our *Nestin^{CreERT2};Baf155$^{fl/+}$* mice, the MBP-positive area and the number of CC1^{+} mature OLs were reduced, while the proportion of IP$_3$R-II/OLIG2-positive committed OPCs was significantly increased, indicating a block in differentiation. To more definitively demonstrate the relevance of BAF155 deficiency in oligodendroglial lineage to ASD-related phenotypes, we employed homozygous knockout mice, a strategy consistent with previous studies of ASD risk genes. We believe our work provides novel evidence linking BAF155 to ASD pathology.

We also acknowledge that the use of homozygous mutants limits the direct translatability of our findings to human patients, who typically carry heterozygous mutations. In the future studies, we will incorporate temporally and spatially restricted knockouts, genotype-phenotype correlation with patient data, and multi-omics analyses to further bridge the gap between mouse models and the human conditions. We have acknowledged this limitation in our Discussion section.

Shi X, Lu C, Corman A, et al. Heterozygous deletion of the autism-associated gene CHD8 impairs synaptic function through widespread changes in gene expression and chromatin compaction. Am J Hum Genet. 2023;110(10):1750-1768.

Busch RM, Srivastava S, Hogue O, et al. Neurobehavioral phenotype of autism spectrum disorder associated with germline heterozygous mutations in PTEN. Transl Psychiatry. 2019;9(1):253.

9. Supplemental Figure 2

1. In Supp figure 2C and 2D, there is a consistent reduction in CC1+ and MAG+ staining in the right quadrant of the images. What is the exact region of the mPFC that is imaged and is this imaging region consistent between animals?

Response: Thanks for the reviewer for the careful reading. These CC1 and MAG staining regions are consistent across animals. This right quadrant which shows a consistent reduction in CC1^{+} and MAG^{+} staining is the are nearer the cortex. Because cortical development occurs later than that of the CC, fewer CC1^{+}/MAG^{+} cells are detected there. Now, we have added the detailed location information to the figure legend. In addition, we have added the low magnification images of CC1 and MAG staining in our **New Figure S2D, S2E**.

10. Where all genotypes of mice given tamoxifen?

Response: We are sorry for the lack of clarity in our manuscript. To ensure comparability between experimental and control mice, all genotypes of mice were given tamoxifen. We have provided this detail in Method section, and highlighted in yellow.

11. There is a growing body of evidence from several ASD mouse models related to myelination defects in ASD. A discussion of this literature in the introduction and/or discussion should be included.

Response: We sincerely appreciate the valuable comment, and agree with the reviewer. Recently, a growing body of evidence from postmortem and animal studies indicate that white matter, particularly myelin, undergoes alteration at different stages of development in individuals with autism. Given the well-established roles of myelin in facilitating nerve impulse conduction, promoting synaptogenesis and fine-tuning intracortical network, it emerges as a potential key player in ASD pathology. However, the certain contribution of altered myelin to the diverse symptomology seen in ASD remained unclear. In this study, we successfully induced ASD-like pathology in an OPC-specific knockout mouse model, and revealed that BAF155 regulates nearly two hundred synaptic genes, which is consistent with the notion that OPC-neuron synaptic contacts contribute to psychiatric disorders. Among these synaptic genes, such as *Gabrg3*, the top listed synaptic gene enriched in OPCs, is associated with altered myelination, accounting for ~10-20% ASD cases. Our findings suggest a complex relationship between all three classical pathways of ASD pathogenesis: chromatin remodeling, synaptic formation, and neural projection. In this context, the primary chromatin remodeling mechanism for regulating OPC-neuron synaptic connection significantly impacts myelin-supported neural functions, thereby triggers the onset of autistic symptoms. We have discussed this point in our manuscript, and highlighted in yellow.

Minor concerns:

- Abstract, line 54: eliminate the period in the middle of the sentence.
- In the results section describing Figure 3F – instead of "in vivo specific regulatory mechanism", maybe refer to this as a non-cell autonomous effect.

Response: We thank the reviewer for this valuable comment. As suggested by the reviewer, we have eliminated the period in the middle of the sentence in the line 54 of Abstract, and corrected the "in vivo specific regulatory mechanism" into "a non-cell autonomous effect".

- Figure 5G – a split y-axis may help to display the data points better.

- Y-axis labels are missing space in "fold change".

Response: Thanks for the reviewer for the careful reading. We have changed these figures accordingly.

- Pg 20, what is meant by "most of these susceptible genes do not reproduce ASD phenotype".

Response: We thank the reviewer for this valuable comment. To make our description more accurate, we have re-written this part: "most of these susceptible genes when examined individually, fail to recapitulate ASD-like phenotypes in animal models, and many of their underlying mechanisms remain unknown". As another reviewer suggested, we moved this part to the Discussion section, and highlighted it in yellow.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We thank the reviewer for his/her help and support.

Reviewer #3 (Remarks to the Author):

Summary:

In this manuscript from Wang et al, the authors investigate the role of the chromatin remodeler BAF155 in oligodendroglial function. They generate a new conditional loss-of-function mouse line, BAF155fl/fl mice, then cross this line to NestinCreERT2 to hit most brain cells, and PDGFRaCreERT2 to remove BAF155 from OPCs specifically. Analysis of these mice showed reduced numbers of mature oligodendrocytes and myelin production (i.e. hypomyelination) especially in white matter as well as mPFC and hippocampus. At the physiological level, loss of OPC-expressed BAF155 decreased excitatory and inhibitory miniatures onto pyramidal neurons in mPFC and hippocampus. Behaviorally, removing BAF155 from OPCs impairs social behaviors, and increases repetitive and anxiety-like behaviors consistent with symptoms of ASD in humans. When the BAF155fl/fl; PDGFRaCreERT2 mouse line was subjected to a demyelination model, OPCs without BAF155 were less capable of differentiating. Chip-seq and DEG analysis of purified committed OPCs revealed gene targets of BAF155 related to synaptic function. They then suggest, based upon structural experiments and Ca2+ imaging, that neuron-OPC

synapses are disrupted by loss of BAF155.

Overall, this is a well-written study with high quality, well-organized, and clear figures. I think the topic of how neurons and OPCs communicate in the brain is highly relevant and timely, and the connection with autism increases interest in the work. I think the paper is a particularly good fit for Nature Communications based upon its strength. I only have two main suggestions, as you will see below, but I feel the suggested experiments are really important to address prior to publication, especially providing more extensive evidence that the newly generated mouse line behaves as expected.

Major comments:

1. This appears to be the first reported publication of this new BAF155fl/fl mouse line. However, perhaps I missed it, but I cannot find in the paper the rigorous validation of the line. How do we know that BAF155 is actually disrupted in these mice? Only exon 4 was floxed, so a truncated protein could still exist. To trust in any of these results, a supplemental figure needs to be added validating (1) normal expression of BAF155 in BAF155fl/fl mice versus wildtype mice of the same background strain when Cre or tamoxifen is absent, and (2) the efficient loss of BAF155 protein from most brain cells in the Nestin^{CreERT2} line and from OPCs specifically in the PDGFRA^{CreERT2} line. These are necessary experiments to interpret the findings of the study.

Response: We thank the reviewer for this valuable comment. Based on the reviewer's suggestion, we have performed the staining of BAF155 in four mouse groups (WT without tamoxifen vs. *Baf155*$^{fl/fl}$ without tamoxifen vs. *PDGFRα*^{CreER}*;Baf155*$^{fl/fl}$ without tamoxifen vs. *PDGFRα*^{CreER}*;Baf155*$^{fl/fl}$ with tamoxifen) to validate the newly generated *Baf155*$^{fl/fl}$ mouse line. As shown in our **New Figure S2B**, BAF155 protein level was significantly decreased in *PDGFRα*^{CreER}*;Baf155*$^{fl/fl}$ with tamoxifen group, when compared to other three groups. Moreover, there were no differences among WT without tamoxifen, *Baf155*$^{fl/fl}$ without tamoxifen and *PDGFRα*^{CreER}*;Baf155*$^{fl/fl}$ without tamoxifen groups. These quantifications validated our new *Baf155*$^{fl/fl}$ mouse line, and confirmed that tamoxifen administration can reduce the BAF155 protein level in OPCs. Additionally, as shown in the **New Figure S2C**, the BAF155/PDGFRα co-staining in *Baf155*$^{fl/fl}$ with tamoxifen and *PDGFRα*^{CreER}*;Baf155*$^{fl/fl}$ with tamoxifen groups validated the efficient BAF155 depletion from OPCs.

Furthermore, to validate the efficient BAF155 depletion from brain cells in the *Nestin*^{CreERT2} line, and from OPCs specifically in the *PDGFRα*^{CreERT2} line, we have performed co-staining of BAF155 with cell type markers: NeuN, Aldh1L1, OLIG2, PDGFRα for brain cells in knockout mice. Our results showed reduced BAF155 protein levels in neural stem-cell-derived neurons, astrocytes, and oligodendroglia, but not in microglia **(New Figure S1C, S1D)**. We have added these results in our manuscript, and highlighted in

yellow.

2. In several places, including lines 354-356, the authors indicate that BAF155 disrupts neuron-OPC synaptic connectivity. To support this conclusion, the authors should assess functional synaptic connectivity between neurons and OPCs using acute slice electrophysiology. In the absence of such an analysis, the claim that BAF155 loss dysregulates neuron-OPC synapses is not fully supported by the data.

Response: We thank the reviewer for this valuable comment. As suggested by the reviewer, we performed whole-cell patch-clamp recordings on YFP+ OPCs from healthy control and BAF155 deficient mice (*Pdgfrα^{CreER};Rosa-YFP vs. Pdgfrα^{CreER};Baf155$^{fl/fl}$;Rosa-YFP*) to assess the neuron-OPC synaptic connectivity **(New Figure S5E)**. For voltage-clamp recordings on OPCs, individual slice was transferred to recording chamber with the external ACSF contains (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 2 CaCl2·2H2O, and 1 MgSO4·7H2O. The patch pipette was injected with internal solution (in mM): 135 Csmethanesulfonate, 8 NaCl, 10 HEPES, 2 Mg2ATP, 0.3 Na3GTP, 0.1 Spermine, 7 phosphocreatine and 0.3 EGTA. AMPAR-mediated EPSCs were recorded at -70 mV, NMDAR-mediated EPSCs were recorded at +40 mV. As stimulation electrode, electrode was inserted in stratum radiatum of CA1 area under a fixed stimulus intensity **(New Figure S5F)**. Then, the AMPA/NMDA current ratio, a critical and widely accepted metric for assessing synaptic maturation and functional strength, was calculated as described previously (see below reference). Because of the technical difficulty of OPC patch-clamp, we were only able to record three OPCs in each group under identical stimulus conditions. However, as shown in our **New Figure S5G**, the AMPA/NMDA current ratio potentially reduced in Baf155-deficient OPCs compared with control OPCs, implying again that BAF155 deletion in OPCs impairs the synaptic communication between OPCs and neurons. We also have included this new result in our Result and Method sections, and have acknowledged this limitation of the OPC patch-clamp in our Discussion section.

Minor comments:

1. A lot of speculation around gene expression results, this should be limited or moved to the discussion.

Response: We thank the reviewer for this suggestion. We have refined this section and moved these statements into our Discission section.

Reviewer #4 (Remarks to the Author):

The study entitled " Chromatin remodeling factor BAF155 coordinates oligodendroglial-neuronal communications linked to regional myelination and autism-like behavioral deficits " by Wang et al. investigates the role of BAF155 in OPCs in the context of ASD. They use mouse models to delete BAF155 in OPCs in mice to show myelination defects, altered synaptic activity and behaviour, and they correlate these features to altered expression of synaptic genes in OPCs. This is overall very interesting and relevant findings, deserving publication in Nat. comm.
I have several comments that might help to make even better the manuscript.

Major comments:
1.) In the graphical abstract as well as in regard to Figure 6 the authors claimed to have studied chromatin accessibility. This is not correct, since BAF155 ChIP signals could correlate with altered accessibility but do not show it. For a mechanistic explanation of the altered transcription, indeed an ATAC survey or nucleosome positioning of/in the BAF155 deleted OPCs might be of relevance. Correlation to the observed changes in gene expression would be of benefit in addition. Please correct also the first sentence referencing to Fig. 6A for the above mentioned reason, that BAF presence it not necessarily correlated to accessibility changes.

Response: We feel extremely grateful for the professional reviewing on our work. Based on the reviewer's suggestion, we have revised the manuscript to correct the sentence regarding chromatin accessibility. In addition, we performed the ATAC assay (the assay for transposase-accessible chromatin) on the OPCs acutely isolated from mPFC and hippocampus. This was done to elucidate the role of BAF155 in the transcription of *Gabrg3, Adnp and Nfia* through regulating chromatin accessibility. As shown in our **New Figure 6A**, the result of ATAC assay revealed significantly higher chromatin accessibility levels of *Gabrg3, Adnp*, and *Nfia* in hippocampal OPCs when compared to cortical OPCs. Therefore, we suggest that BAF155 exerts a stronger regulation on these target genes in the hippocampus. We have added this ATAC assay in our Result and Method sections, and highlighted this information in yellow.

2.) The authors claim that the altered expression of synaptic proteins upon BAF155 deletion leads to impaired OPC-neuron transmission. However, they only show altered Ca-transients in OPCs, or synaptic responses in pyramidal neurons independent from each other. It would be nice to see stimulation of neurons and record altered response in OPCs, which would indeed confirm altered synaptic transmission or communication, as written by the authors. Without this experiments there is the correlation between the

events, but not the transmission per se. As the authors identified specific receptors on the OPC side, the relevance of synaptic transmission through these specific receptors can be strengthened by applying selective inhibitors to the WT condition.

Response: We thank the reviewer for this valuable comment. We think this is an excellent suggestion. In response, we performed whole-cell patch-clamp recordings on YFP+ OPCs from healthy control and Baf155 deficient mice (*Pdgfra*^{CreER}*;Rosa-YFP vs. Pdgfra*^{CreER}*;Baf155*$^{fl/fl}$*;Rosa-YFP*) to assess the neuron-OPC synaptic connectivity **(New Figure S5E)**. For voltage-clamp recordings on OPCs, individual slice was transferred to recording chamber with the external ACSF contains (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 2 CaCl2·2H2O, and 1 MgSO4·7H2O. The patch pipette was injected with internal solution (in mM): 135 Csmethanesulfonate, 8 NaCl, 10 HEPES, 2 Mg2ATP, 0.3 Na3GTP, 0.1 Spermine, 7 phosphocreatine and 0.3 EGTA. AMPAR-mediated EPSCs were recorded at -70 mV, NMDAR-mediated EPSCs were recorded at +40 mV. As stimulation electrode, electrode was inserted in stratum radiatum of CA1 area under a fixed stimulus intensity **(New Figure S5F)**. Then, the AMPA/NMDA current ratio, a critical and widely accepted metric for assessing synaptic maturation and functional strength, was calculated as described previously (see below reference). Because of the technical difficulty of OPC patch-clamp, we were only able to record three OPCs in each group under identical stimulus condition. However, as shown in our **New Figure S5G**, the AMPA/NMDA current ratio was potentially reduced in Baf155-deficient OPCs compared with control OPCs, suggesting that BAF155 deletion in OPCs impairs the synaptic communication between OPCs and neurons. We have added this new result in our Result and Method sections, and we also have acknowledged this limitation of the OPC patch clamp technique in Discussion section.

Although we have identified serval potential receptors on the OPC side, GO enrichment analysis revealed that enrichments in synapse-related genes, not only including both presynaptic and postsynaptic compartments, but also including both GABA receptor genes, such as *Gabrg3, Gabrg2*, and glutamate receptor genes, such as *Gria2, Grm7, Grik2*. These neurotransmitter receptors play a crucial role in various developmental processes and myelination, which is significantly more complex than the primary focus of this study, which is to establish a connection between BAF155 in oligodendroglia and ASD pathogenesis. Nonetheless, we believe it would be beneficial for future studies to manipulate one of these receptors on OPCs (We have another ongoing project focusing on GABRG3 on OPCs right now). This could help demonstrate the role of neuronal activity inputs to OPCs, as well as in OPC-related physiology and pathophysiology. We have acknowledged these limitations in our Discussion section, and highlighted in yellow.

3.) How is the connection between altered chromatin in OPCs, myelination defects in axons, altered Ca transients in OPCs, and altered synaptic protein expression in OPCs? Among the target genes, are there as well myelin affecting genes as a missing functional link? How do the authors explain and validate experimentally the different correlations they observe?

Response: We sincerely appreciate the valuable comment. Recently, a growing body of evidence from postmortem and animal studies indicate that white matter, particularly myelin, undergoes alteration at different stages of development in individuals with autism. Given the well-established roles of myelin in facilitating nerve impulse conduction, promoting synaptogenesis and fine-tuning intracortical network, it emerges as a potential key player in ASD pathology. However, the certain contribution of altered myelin to the diverse symptomology seen in ASD remained unclear. In this study, we successfully induced ASD-like pathology in an OPC-specific knockout mouse model, and revealed that BAF155 regulates nearly two hundred synaptic genes, which is consistent with the notion that OPC-neuron synaptic contacts contribute to psychiatric disorders. Among these synaptic genes, such as *Gabrg3*, the top listed synaptic gene enriched in OPCs, is associated with altered myelination, accounting for ~10-20% ASD cases (see below reference).

To examine the function of OPC-neuron synapses, Ca^{2+} imaging was used to reflect the OPC activity. Meanwhile, the Ca^{2+} dynamics has been documented previously as indicative of oligodendroglial processes that precede retractions from axons (see below reference). Specific patterns of Ca^{2+} transients play different roles in myelin growing. These findings indicate that OPCs deficient in BAF155 exhibit abnormal Ca^{2+} dynamics, which may stem from a weakened synaptic transmission between OPCs and neurons. In the present study, we found that the number of oscillatory peaks per minute were significantly decreased in Baf155-deleted OPCs (Figure 5G-H), Ca^{2+} transients had greater amplitude in OPCs with Baf155 deletion (Figure 5I), and the number of higher frequencies Ca2+ transients, diminished in OPCs lacking BAF155 (Figure 5J). Hence, our results link the OPC-neuron synapse with OPC Ca^{2+} dynamics and myelination.

In present study, our findings may suggest a complex relationship between all three classical pathways of ASD pathogenesis: chromatin remodeling, synaptic formation, and neural projection. In this context, the primary chromatin remodeling mechanism for regulating OPC-neuron synaptic connection significantly impacts myelin-supported neural functions, thereby triggers the onset of autistic symptoms. Right now, we have discussed this point in our manuscript.

Stefansson, H., Meyer-Lindenberg, A., Steinberg, S., Magnusdottir, B., Morgen, K., Arnarsdottir, S., Bjornsdottir, G., Walters, G.B., Jonsdottir, G.A., Doyle, O.M., et al. (2014). CNVs conferring risk of autism

or schizophrenia affect cognition in controls. Nature 505, 361-366. 10.1038/nature12818.

Krasnow, A.M., Ford, M.C., Valdivia, L.E., Wilson, S.W., and Attwell, D. (2018). Regulation of developing myelin sheath elongation by oligodendrocyte calcium transients in vivo. Nat Neurosci 21, 24-28. 10.1038/s41593-017-0031-y.

Baraban, M., Koudelka, S., and Lyons, D.A. (2018). Ca (2+) activity signatures of myelin sheath formation and growth in vivo. Nat Neurosci 21, 19-23. 10.1038/s41593-017-0040-x.

4.) On page 11 the authors start the paragraph with " To investigate why loss of BAF155 impairs OPC differentiation in vivo but not in vitro,..." however, the answer to this question is not given by the experimentation. In addition to this, it is not clear whether the ChIP-seq was done on in vivo cells (as suggested by using purified committed progenitors) or on in vitro cultured cells (as suggested by using medium at day 1 and 5). Please clarify - and resolve the difference between native, i.e. in vivo and cultured cells.

Response: We appreciate this insightful comment. In our study, the ChIP-seq experiments were performed using in vitro cultured OPCs, because isolating sufficient numbers of committed OPCs from brain tissue was too difficult and infeasible in technique. Nevertheless, we don't think that the use of cultured cells compromises our purpose of investigating the underlying mechanism of BAF155 regulating OPC development. In the present study, our data showed that BAF155 depletion impairs OPC differentiation in vivo, whereas the same depletion has no effect on differentiation in vitro. Given BAF155 is required for establishing OPC-neuron synaptic connection, and this connection is essential for OPC differentiation and myelination in the CNS (this conclusion is supported both by our findings and by previous studies from other labs, see references). We therefore propose that the differentiation and myelin defects observed after BAF155 loss arises from the absence of neuronal signaling inputs to OPCs. In the *in vitro* experiment, although the chromatin remodeling system was affected by BAF155 depletion, and synaptic genes changed in cultured committed OPCs (shown in Figure 4), there was no neuronal signaling; consequently, OPC differentiation was not altered. This is why we observed no differences in OPC differentiation, or MBP expression *in vitro*. In fact, we think this finding supports the hypothesis that BAF155 plays a critical role in oligodendroglia development and ASD pathogenesis. Right now, we have refined the related texts in our manuscript, and highlighted in yellow.

Synapses shape oligodendrocyte precursor cell development and predict myelination location. Nat Neurosci. 2024;27(2):217-218.

Moura DMS, Brennan EJ, Brock R, Cocas LA. Neuron to Oligodendrocyte Precursor Cell Synapses: Protagonists in Oligodendrocyte Development and Myelination, and Targets for Therapeutics. Front Neurosci. 2022;15:779125.

5.) " The enrichment analysis of these shared genes such as Ank3 (Ankyrin-3 61), Chl1 (Close Homolog of L1 62), Map1b (Microtubule-associated protein 1b 63), and Mbp 64, indicated their substantial participation in essential biological processes, including neuron projection development, developmental growth, and axon development (Figure 6I)." As the authors claim the substantial participation in essential biological processes, they should integrate these genes in their characterization to establish the missing functional links between their observations. As a proof of principle that these are indeed essential components linking BAF, neurotransmitter signaling and neuronal development, one of them might be used to establish the mechanistic link between OPC BAF functions, impacting myelination, and neuronal function/development.

Response: We appreciate this insightful comment. We have to acknowledge that the present study is not able to address all of these intriguing questions. So far, the genes identified in our work are linked with oligodendroglial and neuronal development, as well as the pathogenesis of ASD. However, proving that they are essential mediators linking BAF155, neurotransmitter signaling, and neuronal development is far more intricate than the primary focus of this study, which is to establish a connection between BAF155 in oligodendroglia and ASD pathogenesis. Nonetheless, we believe this line of inquiry is worthwhile for future studies. We have acknowledged this limitation in our Discussion section.

Minor comments:

Abstract:

1. Through the shortening of information in the Abstract, some statements become unprecise:

"...BAF155, a newly identified chromatin remodeling factor linked to autism susceptibility, is primarily expressed in committed OPCs." BAF155 is not newly identified per se - it is perhaps novel in its implication in autism. Further, BAF155 is a member of ncBAF and nBAF; it has been shown to be active in cortical progenitors during embryonic development (10.1016/j.isci.2018.05.014). Therefore the claim that BAF155 is primarily expressed in OPCs seems superficial. The authors phrase much better in the introduction - here, it is formulated in a misleading way.

Further, the authors used contribute/contributing in the same sentence and inserted a "." instead of "," in the last sentence.

Response: We thank the reviewer for careful reading. We have addressed these issues accordingly and refined our Abstract.

Results:

2. "...stage (Figure 4G). In addition, an extensive and significant enrichments of..." please correct

Response: We thank the reviewer for careful reading. We have corrected this mistake.

3." the reduced number of myelinated axons and increased G-ratios in Baf155 knockout mice at both P14 and 8 weeks of age (Figure 1D)." Please explain G-ratios for a broader audience.

Response: We thank the reviewer for this suggestion. We have provided more detailed information of G-ratio in the result of Figure 1D: "G-ratio is defined as the ratio of an axon's diameter to the overall diameter of its myelinated fiber. It serves as a key metric for evaluating myelin integrity and function; lower G-ratio value reflects thicker myelin sheath and a greater degree of myelination".

4. The authors observed differences for GABA and Glutamatergic neurons (Fig. 1E, 6G, 6H). Please explain these differences in regard to potential neuronal functions and correlate to the electrophysiological findings.

Response: We appreciate the valuable comment. As shown in **Figure 1**, PV+ neurons are a class of fast-spiking GABAergic interneurons that provide inhibitory control in the brain. The significant hypomyelination of PV+ axons in BAF155-deficient mice is likely to desynchronize neural networks. This phenomenon is a well-established pathophysiological feature of ASD and schizophrenia, where dysfunction of PV+ interneurons is a common theme. In **Figure 6**, OPCs exposed to GABA versus glutamate exhibit different gene expression profiles. Serval key genes involved in oligodendroglial development and myelination are differentially regulated. Besides, our results also indicate that OPCs express both GABA and glutamate receptors, as shown in **Figure 4, and 5**. These findings suggest that neuronal activity, mediated by the release of GABA and glutamate, provides instructional cues to OPCs. The distinct genetic programs induced by these two primary neurotransmitters imply a sophisticated signaling "code". Various patterns of signaling "code" could regulate OPCs to differentiate or myelinate in ways that are specifically aligned with the needs of the local circuit. This notion is also supported by previous studies showing that glutamate promotes OPC migration, and at later stages, drives differentiation and myelination; GABA-induced GABAA activation inhibits OPC proliferation and reduces myelin thickness, while GABAB activation

stimulates OPC proliferation and migration (see below reference). The chromatin remodeling factor BAF155 is a key regulator that enables OPCs to accurately interpret these neuronal signals. Its deletion disrupts this signaling "code", leading to inappropriate transcriptional responses. The downregulation of genes associated with synaptic development observed in the DEGs, and histological experiments, as shown in **Figure 4, 5 and 6**, suggests that the ability of OPCs to support neuronal development and maintain synaptic connections is disrupted.

Our new electrophysiological results presented in **New Figure S5E-G**, show a potentially reduced AMPA/NMDA current ratio in BAF155-deficient OPCs, suggesting impaired synaptic transmission from neurons to OPCs. Although we were only able to record from three OPCs in each group under identical stimulus conditions due to the technical difficulties associated with patch-clamping OPCs, this electrophysiological deficit provides a functional link between the molecular changes represented by the DEGs in **Figure 6** and the structural changes, as reflected by myelin deficits in **Figure 1**, and the OPC-neuron synaptic deficits in **Figure 5**. If OPCs can't properly sense neuronal activity due to faulty synapses, they will be unable to execute their developmental program appropriately, including the precise timing and targeting of myelination. We have added this statement in Discussion section and highlighted it in yellow.

As mentioned above in the point 2, although we have identified potential neurotransmitter receptors on the OPC side, these neurotransmitter receptors play a complex role in various developmental processes and myelination, which is beyond the primary focus of this study. However, we do believe it is a very important comment. Our future study will manipulate specific receptors, such as GABRG3 on OPCs This will be a crucial next step to directly test the causal chain, and further clarify the role of neuronal activity in guiding myelination through BAF155 in OPCs. We have discussed this point in our Discussion section, acknowledged these limitations, and highlighted them in yellow.

Braaker, P.N., Mi, X., Soong, D., Bin, J.M., Marshall-Phelps, K., Bradley, S., Benito-Kwiecinski, S., Meng, J., Arafa, D., Richmond, C., et al. (2025). Activity-driven myelin sheath growth is mediated by mGluR5. Nat Neurosci 28, 1213-1225. 10.1038/s41593-025-01956-9.

Chen, T.J., Kula, B., Nagy, B., Barzan, R., Gall, A., Ehrlich, I., and Kukley, M. (2018). In Vivo Regulation of Oligodendrocyte Precursor Cell Proliferation and Differentiation by the AMPA-Receptor Subunit GluA2. Cell Rep 25, 852-861.e857. 10.1016/j.celrep.2018.09.066.

Hamilton, N.B., Clarke, L.E., Arancibia-Carcamo, I.L., Kougioumtzidou, E., Matthey, M., Káradóttir, R., Whiteley, L., Bergersen, L.H., Richardson, W.D., and Attwell, D. (2017). Endogenous GABA controls oligodendrocyte lineage cell number, myelination, and CNS internode length. Glia 65, 309-321. 10.1002/glia.23093.

Luyt, K., Slade, T.P., Dorward, J.J., Durant, C.F., Wu, Y., Shigemoto, R., Mundell, S.J., Váradi, A., and Molnár, E. (2007). Developing oligodendrocytes express functional GABA(B) receptors that stimulate cell proliferation and migration. J Neurochem 100, 822-840. 10.1111/j.1471-4159.2006.04255.x.

5. "neither it affected proliferation OPCs (Figure S2F)." Please correct

Response: We thank the reviewer for careful reading. We have addressed this issue accordingly.

6. In regard to Figures 3 and 6 the authors observed differences in mPFC and hippocampus. If the myelination defect is higher in mPFC compared to the hippocampus, but responses on the targets is higher in hippocampus, it seems that the processes are decoupled? Maybe the authors can explain this?

Response: We thank the reviewer for careful reading and insightful comment. We think there are three main reasons may underlie the regional differences we observed. First, the timing and spatial pattern of myelination differ across brain regions. In mice, hippocampal myelination begins earlier. MBP expression can be detected around P8; whereas the mPFC shows myelin formation around P10. This developmental lag has been reported previously (see reference). While mice complete their myelination within a few weeks, human myelination extends over several years and continues into early adulthood, especially in the prefrontal cortex (see reference). Consequently, in our case, the hippocampus, which may develop earlier, and undergo a longer period of myelin refinement; therefore, the myelination deficit in hippocampus tends to diminish over time, and the myelination deficit in mPFC is larger. Our data shown in Figure 1C also reflects this trend: the myelin deficit at 8 weeks is markedly smaller than that observed at P14.

Second, as shown in our **New Figure 6A-C**, BAF155 exhibits higher chromatin accessibility at *Gabrg3, Adnp* and *Nfia* loci in the hippocampus than in the mPFC, suggesting that region-specific chromatin accessibility of BAF155 may also contribute to regional myelin differences. Moreover, the residual BAF155 signal is still detectable in *PDGFRα*^{CreER}*; BAF155*$^{fl/fl}$ mice. Hence, we suggest that BAF155 more strongly regulates these target genes in the hippocampus, potentially buffering or compensating for the impact of BAF155 loss more effectively, resulting in a milder myelin phenotype compared with the mPFC.

Third, given the myelin formation is highly adaptive progress in the CNS, and the number of myelin segments that each mature OLs can form is different (see reference), along with the variations among different animals, these factors contribute to the different myelin phenotypes observed in different brain regions.

Ozarkar SS, Patel RKR, Vulli T, Friar CA, Burette AC, Philpot BD. Regional analysis of myelin basic protein across postnatal brain development of C57BL/6J mice. Front Neuroanat. 2025;19:1535745.

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol. 2013;106-107:1-16.

Zeiss CJ. Comparative Milestones in Rodent and Human Postnatal Central Nervous System Development. Toxicol Pathol. 2021;49(8):1368-1373.

Benamer N, Vidal M, Balia M, Angulo MC. Myelination of parvalbumin interneurons shapes the function of cortical sensory inhibitory circuits. Nat Commun. 2020;11(1):5151.

7. The origin of several data sets is not clear and need to be referenced by giving the respective numbers of their deposition to publicly accessible databases:

- RNAseq upon GABA and Glutamate
- Chip-seq H3K27Ac and H3K4me3
- Chip-seq of BAF155

Please give the original data source and make sure these are publicly available to the readership.

Response: We thank the reviewer for careful reading. RNAseq upon GABA and Glutamate and ChIP-seq data of BAF155 in this manuscript have been deposited in the NCBI GEO database under the accession number GSE282099 (reviewer token: yfaliyyijhkhdeb) and GSE282098 (reviewer token: wzibcscgdviptqp), respectively. Chip-seq H3K27Ac and H3K4me3 data was from GSM1040161, GSM2225634 and GSM1040162 (see below reference). We have added this information in our manuscript. These data sets are publicly available as of the date of publication.

Ou Z, Sun Y, Lin L, et al. Olig2-Targeted G-Protein-Coupled Receptor Gpr17 Regulates Oligodendrocyte Survival in Response to Lysolecithin-Induced Demyelination. J Neurosci. 2016;36(41):10560-10573.

8."... which result in regional myelin development and contribute to the diverse onset of symptoms associated with ASD." Do the authors refer to local defects in myelination?

Response: Yes. We have refined this word accordingly.

9. Figure legends, Figures:

Please give n numbers for all experiments, even if individual data points are given.

It is a very good way to indicate the p-values in the graphs as done for S1D - why not

doing it consistently for all?

Figure 4: 1d and 5d brains is not understandable; please describe what is used as material correctly. Also give n-numbers and statistics for 4I.

Response: We thank the reviewer for this suggestion. We have provided N numbers and p-values for all figures.

Discussion:

Maybe the authors could elaborate on how the communication between OPC receiving inputs from neurons can be related to abnormal neuronal functions in more detail and by citing the relevant literature regarding the transmitters involved?

What are these transmitters, and how do the authors integrate their findings of interneurons being heavily impacted, and OPCs responding differently to GABA or Glutamate?

Response: We appreciate the reviewer's suggestion, and have expanded the Discussion section about how neuronal inputs to OPCs may link BAF155 deficiency to abnormal neuronal function. OPCs are the only glial cells known to form direct synapses with neurons. Glutamatergic (AMPA/NMDA) and GABAergic ($GABA_A$/$GABA_B$) receptors are expressed on OPC membranes, allowing them to sense activity-dependent release of the two principal CNS neurotransmitters. Glutamate promotes OPC migration, and in later stages, drives differentiation and myelination; excessive activation also can transiently inhibit proliferation. GABA induced $GABA_A$ activation inhibits OPC proliferation and reduces myelin thickness; while $GABA_B$ activation stimulates OPC proliferation and migration. ATP and adenosine regulate intracellular Ca^{2+}, influencing OPC migration and differentiation. Other modulators, such as acetylcholine, serotonin, contribute to region-specific OPC responses during early development. Overall, we suggest that a network effect of neuronal activity on OPCs. If OPCs can't properly sense neuronal activity due to faulty synapses, they will be unable to execute their developmental program appropriately, including the precise timing and targeting of myelination. Besides, OPC-related physiology and pathophysiology, may depend on the balance of excitatory and inhibitory inputs via the specific receptors on OPCs. We have discussed this point in our manuscript, and highlighted in yellow.

Methods:

For the demyelinating mouse model, please precise which age and how long the mice were exposed.

Response: We thank the reviewer for this comment. As suggested, we have added more detailed information about the demyelinating mouse model in the Method section and

highlighted in yellow: “Briefly, for the demyelination mouse model, tamoxifen was administered continuously from P50 to P 54. At P 56, mice were anesthetized by isoflurane (2-3%); after exposing the skull, 1.5μl 1% lysolecithin (Sigma-Aldrich, L0906) was injected into the corpus callosum (1.04 mm lateral and 1.0 mm posterior to the bregma, depth: -1.62 mm). Two weeks later, brain tissue was harvested for immunofluorescence staining and in situ hybridization”.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have adequately addressed our concerns, except for a few minor edits requested below. It is our opinion the manuscript is suitable for publication.

1. Figure legend for supplementary figure one is missing the word supplementary in the legend title.

Response: We thank the reviewer for the careful reading. We have refined the figure legend for supplementary figures one accordingly.

2. Concentrations for TTX, BNQX and AP-5 should be in micromolar not millimolar.
3. Never heard of BNQX, I have heard of CNQX and DNQX.

Response: We thank the reviewer for the careful reading. The concentration of TTX, NBQX and AP-5 is indeed micromolar rather than millimolar, and we have added the information in the Method section.

We sincerely apologize for mistakenly writing NBQX as BNQX. We have already corrected it.

4. Pg. 9, a better explanation for the differing results between sEPSC and mEPSC data would be helpful. It currently states "suggesting that the reduction in spontaneous network activity is attributable to decreased axonal myelination" should also include statements regarding that the no difference in mEPSC frequency and amplitude suggests that synaptic density appears to be intact, and only AP-dependent transmission is affected based on sEPSC data, which indicates mylenation defects may be effection AP-dependent synaptic transmission.
Response: We thank the reviewer for this valuable comment and suggestion. We have refined the text accordingly.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We thank the reviewer for his/her help and support.

Reviewer #3 (Remarks to the Author):

The authors have addressed my concerns. The paper is now suitable for publication in Nat Comms

Response: We thank the reviewer for his/her help and support.

Reviewer #4 (Remarks to the Author):

The authors addressed my comments to enhance their manuscript. There is few things left, that could be added and corrected in the manuscript.

1. Lines 235-236: the authors write about potential enhancer regions. As the H3K27ac data are available and used in the manuscript, I suggest to map the BAF155 binding sites and H3K27ac enrichment and to strengthen the statement, that "enhancers" are bound by BAF155. Presently, without this information, it is more suitable to write about regulatory regions, as intragenic regions are not per se enhancers.

Response: We thank the reviewer for this valuable comment and suggestion. We have refined the text accordingly.

2. Line 260: instead of "specific for H3K27Ac" it would be more suitable to write "enriched for".
3. Line 278: correct "labled"
4. Line 321: correct "play"
5. Line 426: change "triggers" to "triggering"
6. Line 427: change to " in the present study,"
7. Line 430: change "response" to "respond"
8. Line 442: change to "provides an instructional"
9. Line 447: change to "sensing the neuronal"
10. Line 451: change "oligodendroglial lineage" to "oligodendrocytes"
11. Line 460: change to "and humans"

Response: We sincerely appreciate the reviewer for the careful reading. We have addressed these issues accordingly and refined our manuscript.