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Peer Review File

Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction

Corresponding Author: Professor Eunjoon Kim

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)
Seoyeong Kim and colleagues investigate the role of the alternatively spliced mini-exon meB in the LAR family receptor protein tyrosine phosphatase PTPdelta (Ptprd). The authors generate mice expressing Ptprd lacking meB and focus their analysis on heterozygous mice (Ptprd-meB+/-), as they unexpectedly find that Ptprd-meB-/- homozygous mice show impaired postnatal survival. The authors perform an extensive phenotypic analysis of these mice, including behavioral testing, ultrastructural analysis, electrophysiology and (phosphor)proteomics. This approach uncovers a striking example of synaptic specificity: deletion of Ptprd-meB in entorhinal cortex axons has opposing effects on synaptic transmission and density in two target cell populations of these axons: excitatory synaptic transmission and synaptic density are decreased in dentate gyrus granule cells but increased in parvalbumin-expressing interneurons in the dentate gyrus. Database analysis shows that the Ptprd-meB interactor IL1RAP is highly expressed in dentate granule cells, but minimally expressed in interneurons in the dentate gyrus. The authors propose that deletion of Ptprd-meB reduces postsynaptic levels of IL1RAP in synapses of entorhinal axons with dentate granule neurons. In support of this view, their proteomics analysis shows that IL1RAP levels are reduced in the postsynaptic density (PSD) fraction of Ptprd-meB+/- mice. Overall, this study sheds new light on the role of the poorly characterized Ptprd-meB. The experiments are very well performed, the data is convincing, and the text is clearly written. My comments below are minor and meant to further clarify the interpretation of the results.

Minor comments.

1. Page 2, line 38: LAR is Ptprf.
2. Typo: Fig. S3i panel: ‘retireval’ (retrieval)
3. Page 9, line 177: instead of mEPSCs, the authors mean sEPSCs.
4. What happens to protein levels of Ptprs and Ptprf in Ptprd-meB+/- mice?
5. Fig. 2c: why is the effect of Ptprd-meB loss visible in mEPSC frequency, but not in sEPSC frequency? Please explain this more clearly in the text. If network activity compensates, did the authors observe differences in activity of entorhinal output synapses in Ptprd-meB mutant vs wildtype mice?
6. Can the authors show larger magnification of synapses/PSDs in the images in Fig. 3f, g? The synaptic contacts are difficult to see. Related: how was excitatory synaptic density quantified? The authors mention it is difficult to analyze PSD morphology because of its less prominent features in these neurons.
7. Please indicate what white dotted lines are in Fig. 3h. Presumably these indicate the outline of dendritic spines?
8. Page 12, line 258: the authors mean a control experiment without Cre.
9. Please speculate why the only binding partner that shows reduced expression in Ptprd-meB+/- mice is IL1RAP (Fig. 5b-d). Since these are whole brain lysates or fractions obtained from whole brain, would the authors not expect that other binding partners of IL1RAP, such as LRFNs or SLITRKs, are also downregulated? Presumably these are broadly expressed in the mouse brain.
10. Fig. 6b-d: analysis of phosphotyrosine polypeptides shows many changes in Ptprd-meB+/- brains, but relatively few changes are observed in conditional knockouts in which Ptprd-meB is removed from forebrain excitatory neurons using Emx1-Cre, or from inhibitory neurons using Vgat-Cre. Why is this? The authors conclude that global deletion of Ptprd-meB has greater effects than cell type-specific deletion, but the global analysis is done in heterozygote animals and the cell type-

specific analysis is done in homozygous animals (e.g. Emx1-Cre;Ptprd-meBfl/fl). Please elaborate on the interpretation of these results.
11. Please explain the interpretation of the phosphoproteomics analysis in Fig. 6. Differentially expressed protein peptides show a strong postsynaptic component. Proteins that are direct targets of Ptprd would show upregulated phosphorylation following loss of Ptprd-meB; these would be expected to be primarily localized in the presynapse, like Ptprd. Instead, these upregulated polypeptides seem primarily postsynaptic. Why would this be? Downregulated pTyr-polypeptides could be a consequence of loss of trans-synaptic interactions of Ptprd-meB with postsynaptic binding partners.

Reviewer #2

(Remarks to the Author)
Summary
In this manuscript, the authors tried to understand the synaptic role of a specific microexon (MeB) of PTPdelta (a member of LAR-type PTP family proteins). Authors first used previously published single cell datasets and MicroExonator program to calculate levels of meA and meB splice insertions (PSI) in LAR-PTP mRNAs. ~30% of homozygous Ptprd-meB KO mice showed early postnatal lethality, therefore authors used heterozygous Ptprd-meB KO for the remaining experiments, also using heterozygous Ptprd-meA KO for comparison. They then recorded spontaneous excitatory synaptic transmission in dentate gyrus granule cells and interneurons from heterozygous Ptprd-meB KO and found opposite phenotypes. They further performed electron microscopic imaging to observe similar opposite changes in granule cells and parvalbumin-positive interneurons. They then used ChR2-based optogenetics to calculate excitatory-inhibitory ratios in the medial entorhinal cortex-dentate gyrus granule cell synapses. To elucidate possible mechanistic basis for the various phenotypes of heterozygous Ptprd-meB KO, they performed proteomics analysis and found decreased levels of IL1RAP in the postsynaptic density fractions of whole brain lysates. Lastly, they analyzed phosphor-tyrosine proteomic analyses and presented a number of potential molecular changes. Although I admire a large volume of data presented in this manuscript, I cannot recommend its publication in Nature Communications at its current form, partly because most data are too descriptive and because compared to previously published literatures conceptual advancements are relatively limited.

Major comments
1. This study used “whole” heterozygous Ptprd-meB KO for most functional experiments because homozygous counterpart exhibited decreased survival. Surprisingly, authors observed ~2-3-fold higher postnatal lethality in homozygous Ptprd-meB KO than in global Ptprd KO. Although authors provided possible explanations, it is hard to reconcile that deletion of Ptprd meB+ variants produces more severe mouse mortality. Particularly, as most Ptprd variants are expressed as meB+ (Fig. 1), it can be assumed that Ptprd-meB KO is functionally analogous to Ptprd KO, particularly in adult-staged mice. In this regard, most results in this study can be ascribed to deletion of PTPdelta proteins.

2. Authors provided the analyses of single cell RNA-seq data and mentioned that their data were partly consistent with the results from a recent published paper (PMID 38388459). However, the authors did not clearly provide why some discrepancies occurred and why their data (Fig. 1 and Supplementary Fig. 1) are more reliable. The authors did not validate their RNA-seq analyses with other independent experiments, therefore this reviewer was not convinced of the validity of the current results. In addition, the public datasets currently available overall lacked sequencing read depth for reliable microexon analyses, questioning reliability of those data. Authors need to provide more direct evidence to convince the reviewer and possible readers to claim their findings.

3. With the current heterozygous Ptprd meB KO, authors cannot convincingly claim the presynaptic role of PTPdelta meB microexon in regulating EC-DG excitatory synapses, because PTPdelta is expressed in both EC neurons and DG neurons (granule cells and interneurons). In Figure 4, the authors expressed ChR2 specifically MEC neurons but measured optical PSCs in mice lacking PTPdelta meB variants over all brain regions. These are potentially confounding and many of the results reported in this study can be equally ascribed to “postsynaptic” role of PTPdelta meB variants.

4. Authors proposed IL1RAP as a physiologically important ligand for PTPdelta meB variants based on proteomics results. However, loss of synaptic levels of a specific synaptic protein cannot necessarily indicate relative significance than the other ligand proteins (for example, Slitrks and SALMs). Indeed, other proteins (i.e., Slitrk2, Slitrk3, Slitrk5 and SALM5) are expressed with similar patterns as IL1RAP in dentate gyrus (Supplementary Fig. 7). Did the authors monitor the protein expression level of these other candidates?

5. The electrophysiological phenotypes in DG neurons (granule cells vs. interneurons) are intriguing but confusing at most. Why are they different? Authors showed that meB+ variants are equally expressed in both granule cells and interneurons. Can authors test an idea that different PTPDelta meB+-dependent synaptic adhesion events are differentially involved in different cell types? I strongly believe that addressing this question would provide a new insight that can be considered conceptually advanced.

6. In electron microscopic imaging analyses, authors examined EC-DG parvalbumin+ neuron synapses (Fig. 3). In contrast, authors did not discriminate between the interneuron subtypes in the electrophysiological recordings (Fig. 2). Hence, authors cannot directly compare these results and provide additional sets of electron microscopic imaging results that probe the EC-DG somatostatin+ neuron synapses. Moreover, is PTPdelta also expressed in mossy cells? Mossy cells are critical components that regulate the excitation/inhibition ratio in dentate gyrus granule cells. Authors need to provide evidence that PTPdelta meB+ variants expressed in mossy cells are involved in the synaptic regulation of excitation/inhibition.

7. Importantly, authors cannot claim that PTPdelta meB microexon is important for PTPdelta meA microexon, except for the mouse survival that can be explained as similar to global PTPdelta deletion (see PMID 10856223). Simple phenotypic severity cannot be translated as physiologically more significant. Authors claimed that meA microexon inclusion is more prevalent in excitatory neurons (line 384), but this claim seemed to contrast with the results from PMID 38388459 that extensively profiled the meA+ and meB+ inclusion patterns in various cell types of various brain regions. This apparent discrepancy was not thoroughly and compellingly discussed in the current study.

8. Proteomics results are extensive and potentially interesting. However, the physiological significance of their findings were not explored in depth. Considering authors' previous study on LAR-type PTPs and similar analyses (see PMID 32142410), I believe that Nature Communications should now consider more advanced theory and observation than mere descriptive results. For example, what is the physiological significance of phosphor-tyrosine level changes in a variety of synaptic proteins when PTPdelta meB+ variants were deleted?

9. Authors focused on MEC-DG, but not LEC-DG, synapses. Is PTPdelta expressed much higher in MEC axons? Authors need to provide rationale why LEC-DG synapses are not analyzed in the current study.

Minor comments:
1. Introduction (lines 41-42): authors overlooked neurexins and netrin-G1 as putative binding partners for LAR-type PTPs. They should be included with appropriate citations (PMID 23986473, PMID 33037075, PMID 31995730).
2. Introduction (line 63): PMID 38388459 indeed provided evidence to support the PTPdelta meA microexon in regulating specific excitatory synapses and object location memory. Hence, this should be fully acknowledged. Otherwise, authors need to provide more critiques on this paper.
3. Discussion (lines 410-412): How can authors ensure that the "same" EC axons cause opposite changes onto different types of GC neurons? The same argument can be going to IL1RAP (Figures 4 and 5).
4. Are PTPdelta meB-dependent transsynaptic interactions regulated, as similar as PTPdelta meA-dependent pathways? It seems that PTPDelta is primarily expressed as MeB+, hence Slitrks, SALMs and IL1RAP are more germane than Neuroligin3.
5. Female heterozygous Ptprd meB KO mice showed similar behavioral phenotypes as their male counterparts (Supplementary Fig. 3). These results are quite consistent with the previous results (PMID 38388459), hence this point should be mentioned. Moreover, authors need to provide possible reason(s) why the female KO showed weaker anxiety-like behaviors, possibly in Discussion.
6. Throughout the various figure schema, legends and methods, the ages of experimental mice were not clearly indicated.

Reviewer #3

(Remarks to the Author)
NCOMMS-24-31979
Review comment:
Kim et al., "Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction"

Receptor protein tyrosine phosphatase δ (PTPδ) is one of the major presynaptic organizers and is associated with various brain disorders such as attention-deficit hyperactivity disorder (ADHD) and intellectual disability. Trans-synaptic interactions with its postsynaptic partner organizers for inducing synaptic differentiation are regulated by alternatively spliced mini-exons termed meA and meB in PTPδ. The physiological role of PTPδ-meA has been studied using PTPδ-meA-mutant mice while that of meB remains unclear due to the lack of studies on PTPδ-meB-mutant mice. In this paper, Kim et al. investigated spatiotemporal expression patterns of meA and meB splice inserts in Ptprd and then generated PTPδ-meB-lacking mice to explore the physiological role of PTPδ-meB in the levels of behavior, synapse, neural circuit, and molecular phenotype. Overall, every experiment was appropriately performed and the result is explicitly presented. The findings are novel and may attract considerable interest in this research field, although the relationship between the molecular and synaptic/circuit phenotypes appears partly ambiguous. The following points should be addressed before publication.

Major points:
1. It is convincing that the decrease in excitatory synaptic transmission and density in DG-GCs of Ptprd-meB+/– mice is related to the decreased expression of IL1RAP, which is selective to the meB-containing PTPδ variants. Meanwhile, in DG-INs, excitatory synaptic transmission and density were increased in Ptprd-meB+/– mice. The low expression level of IL1RAP in DG-INs can explain why excitatory synaptic transmission and density were not decreased in DG-INs but does not explain why they were increased in DG-INs. As suggested by the authors in the Discussion, one possibility is the involvement of NLGN3, which interacts with meB-lacking PTPδ variants. This could be tested by examining excitatory/inhibitory synaptic ratios in DG-INs of NLGN3-mutant mice.
2. The result of the pTyr proteomic analysis in global Ptprd-meB+/– mice substantially differ from those in two distinct types of conditional Ptprd-meB–/– mice. Why does global PTPδ-meB deletion have greater effects on DEPPs? Also, I could not understand how the changes in the pTyr levels of presynaptic or postsynaptic molecules identified by the proteomic analysis lead to the electrophysiological/histochemical properties of neurons in PTPδ-meB deletion mice. Please clarify this point.

Minor points:
1. The term "density of PSD" indicates the number of spines per 1000 μm2 in this paper but may indicate how much PSD molecules are condensed in spines in a general context. Defining "density of PSD" in the main text would avoid misunderstanding although this is described in Methods.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I thank the authors for convincingly addressing my concerns. The added clarifications and experimental data have further strengthened their interesting manuscript.

Reviewer #2

(Remarks to the Author)
Authors have tried to address the reviewer's various comments by performing additional experiments. I recommend the publication of this revised manuscript in Nature Communications.

Reviewer #3

(Remarks to the Author)
Re:NCOMMS-24-31979A
Kim et al., “Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction”

I appreciate the authors' great efforts to address the reviewers' comments. Meanwhile, I raise the following points to be addressed before publication.

- ln. 315-316, pg 15. "... If this were the case, reducing Neuroligin-3 expression in DG-INs should dampen the heightened excitatory synaptic transmission ... "

Which data correspond to "heightened excitatory synaptic transmission"? The increased mEPSC frequency shown in Fig. 2G? "heightened excitatory synaptic transmission" may be an "increase in excitatory PSD density". Am I correct?

- ln. 322-324, pg 15. "... This finding suggests that increased trans-synaptic PTPδ-Neuroligin-3 interaction at DG-IN excitatory synapses is unlikely to underlie the altered eEPSC/eIPSC ratios in DG-GCs"

Under the knockdown condition in this study, the expression level of Nlgn3 decreased to half the level of the control cell. The remaining Nlgn3 might be enough to form trans-synaptic PTPδ-Neuroligin-3 interaction at DG-IN excitatory synapses and alter eEPSC/eIPSC ratios in DG-GCs. I suppose that the possibility that Nlgn3 is involved in increasing the excitatory PSD density of DG-INs in Ptprd-meB+/– cannot be excluded.

- Regarding pTyr proteomics, it remains unclear how the changes in the pTyr levels of presynaptic or postsynaptic molecules identified by the proteomic analysis are related to the decrease in the excitatory PSD density in DG-GCs and the increase in DG-INs. In this context, I could not understand the role of IL-1β, which is discussed based on the additional experiment exploring p38 signaling cascade. Is this cascade associated with synapse formation or maintenance?

Point-by-point responses to reviewer comments

Re: **Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction**
by Kim, Shin, Kang, Yang, Cho, Paik, Kim, Yi, Lee, Koo, Bok, Bae, Kim, and Kim.

Reviewer #1 (Remarks to the Author):

Seoyeong Kim and colleagues investigate the role of the alternatively spliced mini-exon meB in the LAR family receptor protein tyrosine phosphatase PTPdelta (Ptprd). The authors generate mice expressing Ptprd lacking meB and focus their analysis on heterozygous mice (Ptprd-meB+/-), as they unexpectedly find that Ptprd-meB-/- homozygous mice show impaired postnatal survival. The authors perform an extensive phenotypic analysis of these mice, including behavioral testing, ultrastructural analysis, electrophysiology and (phosphor)proteomics. This approach uncovers a striking example of synaptic specificity: deletion of Ptprd-meB in entorhinal cortex axons has opposing effects on synaptic transmission and density in two target cell populations of these axons: excitatory synaptic transmission and synaptic density are decreased in dentate gyrus granule cells but increased in parvalbumin-expressing interneurons in the dentate gyrus. Database analysis shows that the Ptprd-meB interactor IL1RAP is highly expressed in dentate granule cells, but minimally expressed in interneurons in the dentate gyrus. The authors propose that deletion of Ptprd-meB reduces postsynaptic levels of IL1RAP in synapses of entorhinal axons with dentate granule neurons. In support of this view, their proteomics analysis shows that IL1RAP levels are reduced in the postsynaptic density (PSD) fraction of Ptprd-meB+/- mice. Overall, this study sheds new light on the role of the poorly characterized Ptprd-meB. The experiments are very well performed, the data is convincing, and the text is clearly written. My comments below are minor and meant to further clarify the interpretation of the results.

→ We appreciate the encouraging and insightful comments of the reviewer.

Minor comments.

1. Page 2, line 38: LAR is Ptprf.

→ Corrected. We appreciate the careful comments of the reviewer.

2. Typo: Fig. S3i panel: ‘retireval’ (retrieval)

→ Corrected.

3. Page 9, line 177: instead of mEPSCs, the authors mean sEPSCs.

→ Corrected.

4. What happens to protein levels of Ptprs and Ptprf in Ptprd-meB+/- mice?

→ We checked Ptprs and Ptprf protein levels in the quantitative proteomic analysis data and found that the two proteins were not significantly changed in *Ptprd-meB*$^{+/-}$ mice as compared with WT mice. We created a new supplementary figure (**Supplementary Fig. 7)** showing the changes of whole lysate, SPM, and PSD levels of the LAR family proteins (PTPδ, PTPσ, and LAR).

5. Fig. 2c: why is the effect of Ptprd-meB loss visible in mEPSC frequency, but not in sEPSC frequency? Please explain this more clearly in the text. If network activity compensates, did the authors observe differences in activity of entorhinal output synapses in Ptprd-meB mutant vs wildtype mice?

→ We agree with the reviewer that the decreased mEPSC frequency might have been compensated by network activity, as shown by the normalized sEPSC frequency (**Fig. 2a-c**). Supporting the possibility of presynaptic compensation, the PPR was decreased at MEC-GC synapses (**Supplementary Fig. 6c**). We clarified this in the revised Results.

6. Can the authors show larger magnification of synapses/PSDs in the images in Fig. 3f, g? The synaptic contacts are difficult to see. Related: how was excitatory synaptic density quantified? The authors mention it is difficult to analyze PSD morphology because of its less prominent features in these neurons.

→ We added an electron micrograph showing a larger image of synapse/PSD (an image showing large PSD in the synapse between Pv-positive dendrite and VGLUT1-positive presynaptic axon terminal) in **Fig. 3f**.

These excitatory synapses on Pv-positive neurons quantified as a synapse when VGlut1 signals are apposed to Pv-positive dendrites. Because PSD structures are thin in these excitatory synapses, we did not quantify the morphology of these synapses. We clarified this in the revised Results.

7. Please indicate what white dotted lines are in Fig. 3h. Presumably these indicate the outline of dendritic spines?

→ Yes, white dotted lines indicate the outlines of dendritic spines. We clarified it in the figure legend and the figure panel.

8. Page 12, line 258: the authors mean a control experiment without Cre.

→ Correct. We clarified it in Results.

9. Please speculate why the only binding partner that shows reduced expression in Ptprd-meB+/- mice is IL1RAP (Fig. 5b-d). Since these are whole brain lysates or fractions obtained from whole brain, would the authors not expect that other binding partners of IL1RAP, such as LRFNs or SLITRKs, are also downregulated? Presumably these are broadly expressed in the mouse brain.

→ It is indeed possible that other binding partners of PTPδ such as LRFN5 and SLITRKs could also be downregulated. However, the quantitative proteomic analysis results indicate that total and synaptic levels of these proteins (known binding

partners of PTPδ) are not significantly changed. We now show the total, SPM, and PSD levels of PTPδ-binding partners in a new supplementary figure (**Supplementary Fig. 7**). That said, we acknowledge that changes at individual synapses could be masked by pooling samples from various brain regions, and we have briefly addressed this possibility in the revised Results.

10. Fig. 6b-d: analysis of phosphotyrosine polypeptides shows many changes in Ptprd-meB+/- brains, but relatively few changes are observed in conditional knockouts in which Ptprd-meB is removed from forebrain excitatory neurons using Emx1-Cre, or from inhibitory neurons using Vgat-Cre. Why is this? The authors conclude that global deletion of Ptprd-meB has greater effects than cell type-specific deletion, but the global analysis is done in heterozygote animals and the cell type-specific analysis is done in homozygous animals (e.g. Emx1-Cre;Ptprd-meBfl/fl). Please elaborate on the interpretation of these results.

→ One possible explanation is that the samples were obtained from different brain regions. In *Ptprd-meB*$^{+/-}$ mice, samples were collected from the whole brain (excluding the cerebellum and olfactory bulb), whereas in *Emx1-Cre;Ptprd-meB*$^{fl/fl}$ and *Vgat-Cre;Ptprd-meB*$^{fl/fl}$ mice, samples were derived solely from the cortex and hippocampus. This latter approach leaves the localization and function of PTPRD proteins in non-cortical/hippocampal neurons—which project to these regions and express PTPδ on their axon terminals—largely unaffected.

Another factor could be the cell types in which Ptprd was deleted. Heterozygous deletion suppresses gene expression in both neurons and non-neuronal cells expressing PTPδ, while Emx1- and Vgat-driven deletion affects only a subset of neuronal populations. We briefly discuss these possibilities in the revised Results.

11. Please explain the interpretation of the phosphoproteomics analysis in Fig. 6. Differentially expressed protein peptides show a strong postsynaptic component. Proteins that are direct targets of Ptprd would show upregulated phosphorylation following loss of Ptprd-meB; these would be expected to be primarily localized in the presynapse, like Ptprd. Instead, these upregulated polypeptides seem primarily postsynaptic. Why would this be? Downregulated pTyr-polypeptides could be a consequence of loss of trans-synaptic interactions of Ptprd-meB with postsynaptic binding partners.

→ We agree with the reviewer. Changes in presynaptic proteins—such as the increased pTyr levels in Kv1.4—likely reflect decreased tyrosine phosphatase activity of PTPδ in presynaptic compartments, whereas the altered pTyr levels in postsynaptic proteins are probably due to disrupted trans-synaptic interactions between presynaptic PTPδ and its postsynaptic partners. For instance, we observed a loss of IL1RAP in the mutant PSD fraction (**Fig. 5b**); IL1RAP forms a complex with IL1R1 upon IL-1β binding to IL1R1 to activate p38 [1], implicated in synaptic regulation [2-4].

To directly test whether reduced IL1RAP postsynaptic enrichment in the mutant neurons affects IL-1β-dependent postsynaptic signaling, we treated cultured

neurons with IL-1β and monitored p38 activation—a MAPK that showed decreased phosphorylation in *Ptprd-meB*$^{+/-}$ mice. We found a significant increase in p38 activation in IL-1β-treated *Ptprd-meB*$^{+/-}$ neurons, as compared with untreated WT neurons (**Supplementary Fig. 10a,b**). We suspect that, under normal conditions, the synaptic localization of IL1RAP via its trans-synaptic interaction with PTPδ containing meB may prevent IL1RAP from interacting with IL-1β-bound IL1R1 and thus suppress p38 activation (**Supplementary Fig. 10c**), although further studies are needed to further explore related mechanisms. We presented this new data (**Supplementary Fig. 10**) and discussed the results in the revised Discussion.

Reviewer #2 (Remarks to the Author):

Summary
In this manuscript, the authors tried to understand the synaptic role of a specific microexon (MeB) of PTPdelta (a member of LAR-type PTP family proteins). Authors first used previously published single cell datasets and MicroExonator program to calculate levels of meA and meB splice insertions (PSI) in LAR-PTP mRNAs. ~30% of homozygous Ptprd-meB KO mice showed early postnatal lethality, therefore authors used heterozygous Ptprd-meB KO for the remaining experiments, also using heterozygous Ptprd-meA KO for comparison. They then recorded spontaneous excitatory synaptic transmission in dentate gyrus granule cells and interneurons from heterozygous Ptprd-meB KO and found opposite phenotypes. They further performed electron microscopic imaging to observe similar opposite changes in granule cells and parvalbumin-positive interneurons. They then used ChR2-based optogenetics to calculate excitatory-inhibitory ratios in the medial entorhinal cortex-dentate gyrus granule cell synapses. To elucidate possible mechanistic basis for the various phenotypes of heterozygous Ptprd-meB KO, they performed proteomics analysis and found decreased levels of IL1RAP in the postsynaptic density fractions of whole brain lysates. Lastly, they analyzed phospho-tyrosine proteomic analyses and presented a number of potential molecular changes. Although I admire a large volume of data presented in this manuscript, I cannot recommend its publication in Nature Communications at its current form, partly because most data are too descriptive and because compared to previously published literatures conceptual advancements are relatively limited.

→ We appreciate the encouraging and insightful comments of the reviewer and tried our best to address the review points and improve the manuscript.

Major comments

1. This study used “whole” heterozygous Ptprd-meB KO for most functional experiments because homozygous counterpart exhibited decreased survival. Surprisingly, authors observed ~2-3-fold higher postnatal lethality in homozygous Ptprd-meB KO than in global Ptprd KO. Although authors provided possible

explanations, it is hard to reconcile that deletion of Ptprd meB+ variants produces more severe mouse mortality. Particularly, as most Ptprd variants are expressed as meB+ (Fig. 1), it can be assumed that Ptprd-meB KO is functionally analogous to Ptprd KO, particularly in adult-staged mice. In this regard, most results in this study can be ascribed to deletion of PTPdelta proteins.

→ The reviewer's point is well-taken in that the meB exon is an integral part of the full-length PTPδ protein. However, we emphasize that the total levels of PTPδ are comparable between WT and *Ptprd-meB*$^{+/-}$ mice, as confirmed by immunoblot analyses using two different antibodies (targeting both the N- and C-terminal regions; **Fig. 1e**) and by quantitative proteomic analyses, which also show no changes in the levels of other family members (PTPσ and LAR; **Supplementary Fig. 7**). Thus, the observed phenotypes in *Ptprd-meB*$^{+/-}$ mice are likely not due to a reduction in overall PTPδ protein but rather to the loss of meB-dependent interactions between presynaptic PTPδ and its postsynaptic binding partners.

Regarding why PTPδ-meB deletion has a stronger impact on neonatal survival compared to global PTPδ deletion, one possibility is that global deletion may induce compensatory changes in related proteins—such as an increase in PTPσ, as previously reported in the PTPδ-mutant hippocampus [5]—whereas the selective deletion of the meB exon does not trigger such compensatory increases. Given that the meB-containing isoform is the predominant form of PTPδ in vivo [6-8], its deletion in neonates may specifically impair the formation of synapses that are essential functions such as feeding. Newborn mammals rely on properly formed brainstem and spinal circuits—for instance, those mediating suckling reflexes and controlling cranial nerve motor pools—to establish functional synapses shortly after birth. If PTPδ-meB is required for connecting these circuits (e.g., by promoting presynaptic differentiation onto motor neurons that innervate the jaw and tongue, or onto hypothalamic feeding centers), its absence could lead to failure of neonates to nurse, ultimately resulting in early postnatal death. These points have been incorporated into the revised Discussion.

2. Authors provided the analyses of single cell RNA-seq data and mentioned that their data were partly consistent with the results from a recent published paper (PMID 38388459). However, the authors did not clearly provide why some discrepancies occurred and why their data (Fig. 1 and Supplementary Fig. 1) are more reliable. The authors did not validate their RNA-seq analyses with other independent experiments, therefore this reviewer was not convinced of the validity of the current results. In addition, the public datasets currently available overall lacked sequencing read depth for reliable microexon analyses, questioning reliability of those data. Authors need to provide more direct evidence to convince the reviewer and possible readers to claim their findings.

→ While we strived to generate high-quality data from publicly available databases, we acknowledge that our dataset is heterogeneous and varies in depth. Although our overall findings are consistent with those of a recent study [8], which supports our conclusions, we believe that our data should not serve as a primary reference given

the availability of more rigorously validated experimental results. Consequently, we have removed the MicroExonator data from the revised manuscript, as it is redundant with previously published work and does not constitute the main focus of our study.

3. With the current heterozygous Ptprd meB KO, authors cannot convincingly claim the presynaptic role of PTPdelta meB microexon in regulating EC-DG excitatory synapses, because PTPdelta is expressed in both EC neurons and DG neurons (granule cells and interneurons). In Figure 4, the authors expressed ChR2 specifically MEC neurons but measured optical PSCs in mice lacking PTPdelta meB variants over all brain regions. These are potentially confounding and many of the results reported in this study can be equally ascribed to “postsynaptic” role of PTPdelta meB variants.

→ It is true that *Ptprd* mRNAs are detected in both presynaptic entorhinal cortex (EC) neurons and postsynaptic dentate gyrus (DG) neurons (**Supplementary Fig. 8**). However, our data indicate that PTPδ protein expression is stronger in EC neurons relative to DG neurons. For example, our previous study demonstrated that PTPδ-tdTomato signals in the stratum lacunosum-moleculare (SLM) of the hippocampus are primarily presynaptic, as supported by EM analysis [5]. The EM images in the present study indicate that PTPδ-tdTomato signals in the ventral hippocampus are stronger in presynaptic axon terminals in the DG region (**Fig. 3h,i**). Importantly, PTPδ-tdTomato signals are largely absent in the mossy fiber tract in the CA3 region which originate from DG-GCs (**Supplementary Fig. 4c**), suggesting that PTPδ expression in DG granule cells is minimal. Additionally, an acute deletion of PTPδ-meB in the medial entorhinal cortex (MEC), but not in the DG, in *Ptprd-meB*$^{+/fl}$ mice recapitulates the synaptic phenotype observed in the MEC-DG-GC pathway of *Ptprd-meB*$^{+/-}$ mice (**Fig. 4g-i**), further supporting that the synaptic changes observed following adult-brain PTPδ-meB deletion stem from a presynaptic loss of PTPδ-meB. These points have been clarified in the revised Results.

4. Authors proposed IL1RAP as a physiologically important ligand for PTPdelta meB variants based on proteomics results. However, loss of synaptic levels of a specific synaptic protein cannot necessarily indicate relative significance than the other ligand proteins (for example, Slitrks and SALMs). Indeed, other proteins (i.e., Slitrk2, Slitrk3, Slitrk5 and SALM5) are expressed with similar patterns as IL1RAP in dentate gyrus (Supplementary Fig. 7). Did the authors monitor the protein expression level of these other candidates?

→ Other postsynaptic binding partners of PTPδ could also contribute the excitatory synaptic loss at the MEC-GC pathway. However, the levels of these proteins (putative binding partners of PTPδ other than IL1RAP) were not changed in any of the proteomic fractions (whole lysate, SPM, and PSD) (**Supplementary Fig. 7**). This however does not exclude the possibility that other PTPδ partners play important roles, considering that the proteome data are derived from multiple cell types. We clarified this in the revised Results.

5. The electrophysiological phenotypes in DG neurons (granule cells vs.

interneurons) are intriguing but confusing at most. Why are they different? Authors showed that meB+ variants are equally expressed in both granule cells and interneurons. Can authors test an idea that different PTPDelta meB+-dependent synaptic adhesion events are differentially involved in different cell types? I strongly believe that addressing this question would provide a new insight that can be considered conceptually advanced.

→ We appreciate the insightful comment. One potential explanation for the increased excitatory synaptic transmission on DG interneurons is that disruption of PTPδ–IL1RAP trans-synaptic interactions in the MEC-to-DG-GC pathway may prevent MEC axon terminals from forming excitatory synapses with DG-GCs, leading them instead to form ectopic synapses with DG-INs. In this scenario, an enhanced interaction between presynaptic meB-lacking PTPδ and postsynaptic NLGN3—which preferentially binds to meB-lacking PTPδ [9]—might occur. However, acute knockdown of neuroligin-3 in the *Ptprd-meB*$^{+/-}$ vDG did not affect eEPSC/eIPSC ratios in DG-GCs, although the baseline difference (decreased eEPSC/eIPSC ratios in control shRNA-treated mutant neurons) could be reproduced in this context (**Fig. 5k–m; Supplementary Fig. 9**), suggesting that the increased excitatory synaptic transmission on DG-INs likely arises from mechanisms that remain to be identified.

6. In electron microscopic imaging analyses, authors examined EC-DG parvalbumin+ neuron synapses (Fig. 3). In contrast, authors did not discriminate between the interneuron subtypes in the electrophysiological recordings (Fig. 2). Hence, authors cannot directly compare these results and provide additional sets of electron microscopic imaging results that probe the EC-DG somatostatin+ neuron synapses. Moreover, is PTPdelta also expressed in mossy cells? Mossy cells are critical components that regulate the excitation/inhibition ratio in dentate gyrus granule cells. Authors need to provide evidence that PTPdelta meB+ variants expressed in mossy cells are involved in the synaptic regulation of excitation/inhibition.

→ We appreciate this comment but have to point out that the cell bodies and dendrites of somatostatin (SST)-expressing neurons in the DG (i.e., HIPP and HIL types) are localized in the hilus region, whereas only their axons are localized in the molecular layer [10], suggesting that these SST neurons are unlikely to receive excitatory synaptic inputs from entorhinal axons. In addition, our data indicate that the hilus region of the DG largely lacks PTPδ-tdTomato signals (**Supplementary Fig. 4c**). In addition, DG-SST neurons have recently been shown to be not the primary mediators of DG-GC inhibition [11]. In contrast, parvalbumin (Pv)-expressing neurons, which reside within (or adjacent to) the granule cell layer and receive monosynaptic excitatory inputs from the entorhinal cortex [12], have been identified a key regulator of DG-GC inhibition and synaptic plasticity [11,13]. Additionally, we could not identify commercial SST antibodies that can visualize SST neuronal dendrites, unlike the Pv antibodies that could visualize the dendrites for PTPδ-tdTomato signals. These results/considerations discouraged us to explore PTPδ-meB functions in SST neurons.

Regarding mossy cells, our data reveal that PTPδ-tdTomato signals are largely absent in the inner molecular layer of the DG (**Supplementary Fig. 4c**)—areas rich in mossy-cell synaptic inputs onto GCs—suggesting that PTPδ is unlikely to be expressed in mossy cells and regulate the mossy cell-GC pathway. This also discouraged us to pursue mossy cell-related PTPδ-meB functions. However, there has been a report showing axons of dorsal DG mossy cells projecting to ventral DG area have diffuse nerve terminals in the middle molecular layer [14], suggesting that we cannot exclude the possibility that dorsal PTPδ-meB-positive mossy cell terminals regulate ventral DG-GCs. We commented on these points on SST neurons and mossy cells in the revised Results.

7. Importantly, authors cannot claim that PTPdelta meB microexon is important for PTPdelta meA microexon, except for the mouse survival that can be explained as similar to global PTPdelta deletion (see PMID 10856223). Simple phenotypic severity cannot be translated as physiologically more significant. Authors claimed that meA microexon inclusion is more prevalent in excitatory neurons (line 384), but this claim seemed to contrast with the results from PMID 38388459 that extensively profiled the meA+ and meB+ inclusion patterns in various cell types of various brain regions. This apparent discrepancy was not thoroughly and compellingly discussed in the current study.

→ We agree that we should be careful in assessing the relative importance of meB versus meA requires considering multiple aspects of the phenotypes. Accordingly, we have removed the Discussion section comparing meB and meA-mutant mice. In addition, we have revised a relevant sentence in the abstract from “Therefore, PTPδ-meB is more important than PTPδ-meA for survival, synaptic, and behavioral phenotypes and regulates excitatory synapses in cell-type-specific and IL1RAP-dependent manners.” to “Therefore, PTPδ-meB is important for survival, synaptic, and behavioral phenotypes and regulates excitatory synapses in cell-type-specific and IL1RAP-dependent manners.”

8. Proteomics results are extensive and potentially interesting. However, the physiological significance of their findings were not explored in depth. Considering authors’ previous study on LAR-type PTPs and similar analyses (see PMID 32142410), I believe that Nature Communications should now consider more advanced theory and observation than mere descriptive results. For example, what is the physiological significance of phospho-tyrosine level changes in a variety of synaptic proteins when PTPdelta meB+ variants were deleted?

→ We agree on the need to pursue the physiological consequences of the altered synaptic enrichment and phosphorylation levels of these proteins—ideally at the level of individual cell types and synapses. As a part of the effort to address this question, we examined whether changes in the trans-synaptic PTPδ-IL1RAP interaction might affect IL-1β signaling, wherein IL-1β binds to IL1R1 (a cognate receptor of IL-1β), which is known to form a complex with IL1RAP to stimulate downstream signaling such as p38 activation in neurons [1]. To this end, we treated cultured hippocampal neurons with IL-1β and monitored p38 activation/phosphorylation and found that p38 activation is abnormally increased in

IL-1β-treated *Ptprd-meB*$^{+/-}$ neurons, as compared with untreated WT neurons (**Supplementary Fig. 10a-c**). This suggests that trans-synaptic PTPδ-IL1RAP interaction regulates IL-1β signaling in neurons. We commented on the results in the revised Discussion.

9. Authors focused on MEC-DG, but not LEC-DG, synapses. Is PTPdelta expressed much higher in MEC axons? Authors need to provide rationale why LEC-DG synapses are not analyzed in the current study.

→ PTPδ-tdTomato signals are comparable in the middle molecular layer and outer molecular layers (**Supplementary Fig. 4c**), which receive synaptic inputs from the MEC and LEC regions of the entorhinal cortex, respectively. This suggests that MEC and LEC neurons express similar levels PTPδ proteins and similarly regulate DG-GCs.

To address this comment more directly, we conducted additional experiments to determine whether eEPSC/eIPSC ratios are altered in the LEC-DG-GC pathway in *Ptprd-meB*$^{+/-}$ mice. We observed no differences in eEPSC/eIPSC ratios between the mutant and WT pathways (**Supplementary Fig. 6f**). This result indicates that the LEC-DG-GC and MEC-DG-GC pathways have distinct PTPδ-meB–related functions, paralleling the previously reported functional differences between these regions for LRRTM3 [15]. We briefly commented on this result in the revised Results.

Minor comments:

1. Introduction (lines 41-42): authors overlooked neurexins and netrin-G1 as putative binding partners for LAR-type PTPs. They should be included with appropriate citations (PMID 23986473, PMID 33037075, PMID 31995730).

→ We cited these references in the revised Introduction.

2. Introduction (line 63): PMID 38388459 indeed provided evidence to support the PTPdelta meA microexon in regulating specific excitatory synapses and object location memory. Hence, this should be fully acknowledged. Otherwise, authors need to provide more critiques on this paper.

→ We commented on this in the revised Introduction.

3. Discussion (lines 410-412): How can authors ensure that the “same” EC axons cause opposite changes onto different types of GC neurons? The same argument can be going to IL1RAP (Figures 4 and 5).

→ We agree and changed “same EC axons” to “EC axons” in the revised Discussion. We also clarified in the legends for Figures 4a and 5j that it remains unknown whether the same axon provides excitatory synaptic inputs onto both DG-GCs and DG-INs.

4. Are PTPdelta meB-dependent transsynaptic interactions regulated, as similar as PTPdelta meA-dependent pathways? It seems that PTPDelta is primarily expressed as MeB+, hence Slitrks, SALMs and IL1RAP are more germane than Neuroligin3.

→ We agree and commented on these aspects in the revised Discussion.

5. Female heterozygous Ptprd meB KO mice showed similar behavioral phenotypes as their male counterparts (Supplementary Fig. 3). These results are quite consistent with the previous results (PMID 38388459), hence this point should be mentioned. Moreover, authors need to provide possible reason(s) why the female KO showed weaker anxiety-like behaviors, possibly in Discussion.

→ We commented in the revised results section that our results (lack of male-female difference in meB-mutant mice) are similar to the previously reported lack of male-female difference in meA expression patterns in the brain [8].

Possible reasons for the lack of sexually dimorphic behaviors in meB-mutant mice remain to be studied, although anxiety-modulatory sex-specific factors (i.e., chromosome/hormone) might be involved [16-19]. We briefly commented on these aspects in the revised Results (not Discussion) because our current data do not support any specific hypotheses regarding these mechanisms.

6. Throughout the various figure schema, legends and methods, the ages of experimental mice were not clearly indicated.

→ We made sure in the revised manuscript that the descriptions of mouse ages are clearly indicated in the figure schema, legends, and methods.

Reviewer #3 (Remarks to the Author):

NCOMMS-24-31979

Review comment:

Kim et al., “Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction”

Receptor protein tyrosine phosphatase δ (PTPδ) is one of the major presynaptic organizers and is associated with various brain disorders such as attention-deficit hyperactivity disorder (ADHD) and intellectual disability. Trans-synaptic interactions with its postsynaptic partner organizers for inducing synaptic differentiation are regulated by alternatively spliced mini-exons termed meA and meB in PTPδ. The physiological role of PTPδ-meA has been studied using PTPδ-meA-mutant mice while that of meB remains unclear due to the lack of studies on PTPδ-meB-mutant mice. In this paper, Kim et al. investigated spatiotemporal expression patterns of meA and meB splice inserts in Ptprd and then generated PTPδ-meB-lacking mice to explore the physiological role of PTPδ-meB in the levels of behavior, synapse, neural circuit, and molecular phenotype. Overall, every experiment was appropriately performed and the result is explicitly presented. The findings are novel and may attract considerable interest in this research field, although the relationship between the molecular and synaptic/circuit phenotypes appears partly ambiguous. The following points should be addressed before publication.

→ We appreciate the encouraging and insightful comments of the reviewer.

Major points:

1. It is convincing that the decrease in excitatory synaptic transmission and density in DG-GCs of Ptprd-meB+/– mice is related to the decreased expression of IL1RAP, which is selective to the meB-containing PTPδ variants. Meanwhile, in DG-INs, excitatory synaptic transmission and density were increased in Ptprd-meB+/– mice. The low expression level of IL1RAP in DG-INs can explain why excitatory synaptic transmission and density were not decreased in DG-INs but does not explain why they were increased in DG-INs. As suggested by the authors in the Discussion, one possibility is the involvement of NLGN3, which interacts with meB-lacking PTPδ variants. This could be tested by examining excitatory/inhibitory synaptic ratios in DG-INs of NLGN3-mutant mice.

→ We appreciate the insightful comment. In response, we acquired Nlgn3-R451C-mutant mice [20]. However, breeding and preparing these mice for experiments has taken longer than anticipated, although we tried our best for the last seven months.

As an alternative approach, we used shRNA to knock down neuroligin-3 in the *Ptprd-meB*$^{+/-}$ dentate gyrus (DG) region. We first assessed the eEPSC/eIPSC ratios in DG granule cells (DG-GCs), hypothesizing that decreasing neuroligin-3 levels in DG interneurons would restore the excitation/inhibition balance in DG-GCs. However, we could not observe shNlgn3-induced changes in eEPSC/eIPSC ratios in DG-GCs from *Ptprd-meB*$^{+/-}$ mice injected with shNlgn3 in the DG (**Fig. 5k-m; Supplementary Fig. 9**), suggesting that the increased excitatory synaptic transmission onto DG interneurons likely results from neuroligin-3-independent mechanisms that remain to be identified. We commented on the results in the revised Discussion.

2. The result of the pTyr proteomic analysis in global Ptprd-meB+/– mice substantially differ from those in two distinct types of conditional Ptprd-meB–/– mice. Why does global PTPδ-meB deletion have greater effects on DEPPs? Also, I could not understand how the changes in the pTyr levels of presynaptic or postsynaptic molecules identified by the proteomic analysis lead to the electrophysiological/histochemical properties of neurons in PTPδ-meB deletion mice. Please clarify this point.

→ One possible explanation is that the samples were obtained from different brain regions. In *Ptprd-meB*$^{+/-}$ mice, samples were collected from the whole brain (excluding the cerebellum and olfactory bulb), whereas in *Emx1-Cre;Ptprd-meB*$^{fl/fl}$ and *Vgat-Cre;Ptprd-meB*$^{fl/fl}$ mice, samples were derived solely from the cortex and hippocampus. This latter approach leaves the localization and function of PTPRD proteins in non-cortical/hippocampal neurons—which project to these regions and express PTPδ on their axon terminals—largely unaffected. Another factor could be the cell types in which Ptprd-meB was deleted. Heterozygous deletion suppresses gene expression in both neurons and non-neuronal cells expressing PTPδ, while Emx1- and Vgat-driven deletion affects only a subset of neuronal populations. We briefly discuss these possibilities in the revised Results.

We agree on the need to pursue the physiological consequences of the altered synaptic enrichment and phosphorylation levels of these proteins—ideally at the level of individual cell types and synapses. As a part of the effort to address this question, we examined whether changes in the trans-synaptic PTPδ-IL1RAP interaction might affect IL-1β signaling, wherein IL-1β binds to IL1R1 (a cognate receptor of IL-1β), which is known to promote the conformational change of IL1R1 to form a complex with IL1RAP and stimulate downstream signaling such as p38 activation in neurons [1]. To this end, we treated cultured hippocampal neurons with IL-1β and monitored p38 activation and found that p38 activation is abnormally increased in IL-1β-treated *Ptprd-meB*$^{+/-}$ neurons, as compared with untreated WT neurons (**Supplementary Fig. 10a-c**). This suggests that the trans-synaptic PTPδ-IL1RAP interaction regulates IL-1β signaling in neurons. We commented on the results in the revised Discussion.

Minor points:

1. The term "density of PSD" indicates the number of spines per 1000 μm2 in this paper but may indicate how much PSD molecules are condensed in spines in a general context. Defining "density of PSD" in the main text would avoid misunderstanding although this is described in Methods.

→ Thanks. We defined the “density of PSD” in the main text properly.

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Re: **NCOMMS-24-31979A**
Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction
by Kim, Shin, Kang, Yang, Cho, Paik, Kim, Yi, Lee, Koo, Bok, Bae, Kim, and Kim.

Point-by-point responses to reviewers' comments

Reviewers' comments

Reviewer #1 (Remarks to the Author):
I thank the authors for convincingly addressing my concerns. The added clarifications and experimental data have further strengthened their interesting manuscript.

→ We appreciate your insightful and constructive comments.

Reviewer #2 (Remarks to the Author):
Authors have tried to address the reviewer's various comments by performing additional experiments. I recommend the publication of this revised manuscript in Nature Communications.

→ We appreciate your insightful and constructive comments.

Reviewer #3 (Remarks to the Author):
Re:NCOMMS-24-31979A
Kim et al., "Alternatively spliced mini-exon B in PTPδ regulates excitatory synapses through cell-type-specific trans-synaptic PTPδ-IL1RAP interaction"
I appreciate the authors' great efforts to address the reviewers' comments. Meanwhile, I raise the following points to be addressed before publication.

→ Thank you for your thoughtful and constructive feedback. We have addressed your three remaining review comments as detailed below.

1. ln. 315-316, pg 15. "... If this were the case, reducing Neuroligin-3 expression in DG-INs should dampen the heightened excitatory synaptic transmission ... "
Which data correspond to "heightened excitatory synaptic transmission"? The increased mEPSC frequency shown in Fig. 2G? "heightened excitatory synaptic transmission" may be an "increase in excitatory PSD density". Am I correct?

→ We agree with the reviewer's comment. Accordingly, we have revised the text by replacing "heightened excitatory synaptic transmission" with "increase in excitatory PSD density" to more accurately reflect our findings.

2. ln. 322-324, pg 15. "... This finding suggests that increased trans-synaptic PTPδ-Neuroligin-3 interaction at DG-IN excitatory synapses is unlikely to underlie the altered eEPSC/eIPSC ratios in DG-GCs"
Under the knockdown condition in this study, the expression level of Nlgn3 decreased to half the level of the control cell. The remaining Nlgn3 might be enough to form trans-synaptic PTPδ-Neuroligin-3 interaction at DG-IN excitatory synapses and alter eEPSC/eIPSC ratios in DG-GCs. I suppose that the possibility that Nlgn3 is involved in increasing the excitatory PSD density of DG-INs in Ptprd-meB+/– cannot be excluded.

→ We agree and have incorporated the following statement into the revised manuscript: "Although we cannot exclude the possibility that the remaining half of the Neuroligin-3 protein, which escaped knockdown, contributes to the increase in excitatory PSD density in DG-INs of *Ptprd-meB*$^{+/-}$ mice."

3. Regarding pTyr proteomics, it remains unclear how the changes in the pTyr levels of presynaptic or postsynaptic molecules identified by the proteomic analysis are related to the decrease in the excitatory PSD density in DG-GCs and the increase in DG-INs. In this context, I could not understand the role of IL-1β, which is discussed based on the additional experiment exploring p38 signaling cascade. Is this cascade associated with synapse formation or maintenance?

→ p38 is known not only to regulate excitatory synaptic plasticity but also to maintain excitatory synaptic structural integrity through the IL-1β–p38 signaling cascade. Specifically, IL-1β enhances p38 activation, subsequently triggering the activation of MK2/3, LIMK, and cofilin. Activated cofilin then promotes actin depolymerization, leading to dendritic spine shrinkage. Consequently, abnormally elevated p38 activation observed in IL-1β treated PTPδ-meB mutant neurons may intensify actin depolymerization, ultimately causing a loss of excitatory postsynaptic density (PSD) structures. We have incorporated these points into our discussion with appropriate references to better illustrate the implications of IL-1β- and p38-related data.

We acknowledge the difficulty in fully elucidating how pTyr proteomic alterations induced by PTPδ-meB deletion contribute directly to excitatory synapse disruption. Nonetheless, we focused specifically on pTyr alterations linked to IL1RAP, given its pronounced reduction at excitatory synapses and its relationship with the IL-1β-p38 signaling pathway, a known regulator of synaptic actin dynamics and excitatory synapse density. We emphasize that these mechanisms are speculative at present and require further detailed investigation. This limitation is now clearly stated within our revised discussion section concerning p38 signaling.