nature portfolio

Peer Review File

Single-Nucleus Analysis of the Adult Human Olfactory Epithelium Uncovers Shared Neurogenesis Programs with the Brain

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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)
The authors collected snRNA-seq data from the human olfactory epithelium (OE) of six living adult donors and integrated an independent dataset from four additional adults to assess the molecular profiles of olfactory sensory neurons (OSNs). Overall, the manuscript is well-written, presents a comprehensive analysis, and provides rich datasets of potential value to the field. The manuscript could be further improved by enhancing methodological clarity and rigor in data presentation. For example:

1. Figure 1i
-- The method used to generate Figure 1i is not immediately clear, as it is described in the middle of the Methods section rather than aligned with the Results presentation. A direct citation to the relevant Methods subsection would be helpful.
-- The cutoff for statistical significance should be explicitly stated. A less stringent cutoff was used for this analysis.
-- The rationale for selecting particular GWAS summary statistics should be provided. For instance, the authors use unpublished schizophrenia (SZ) GWAS data with a smaller sample size, rather than the larger, published PGC SZ dataset (Nature, 2022). Justification for this choice is needed.

2. Figure 2f
-- The criteria for selecting enriched GO terms shown in the figure are unclear. It is understandable that not all significant terms can be shown in the main figure. However, according to Table S3, the subset presented in Fig. 2f does not correspond to the exact top three results. Clear selection criteria are needed to ensure objectivity and reproducibility.

3. Figure 3
-- Figure 3a: The figure presents scaled correlation coefficients rather than raw Spearman values. Since raw correlation coefficients provide interpretable measures of effect size and strength, they should be shown to allow readers to assess the results more transparently.
-- Figure 3b: It is unclear how the gene module score was calculated. Additional methodological detail should be provided.

Minor suggestions
Citing the following representative studies employing olfactory epithelium–derived cellular models for brain disorder research would be helpful in strengthening the motivation and conclusions of this work:

https://pubmed.ncbi.nlm.nih.gov/36291125/
https://pubmed.ncbi.nlm.nih.gov/36379214/
https://pubmed.ncbi.nlm.nih.gov/37250419/

(Remarks on code availability)
The codes are well-organized. It would be helpful if the input datasets corresponding to each script could be shared once the

paper is accepted.

Reviewer #2

(Remarks to the Author)
In this manuscript, Song and colleagues compared human olfactory epithelium cells including GBCs, iOSNs, mOSNs with cortical excitatory neurons based on the snRNA-Seq and reported scRNA-Seq datasets. They reported the convergence between OSNs and CENs, with shared and similar expression pattern genes related with psychiatric disorders and autism. Although authors showed some bioinformatic data, it is not fully well-grounded why the cortical neurons in the CNS and OSNs in the PNS were compared, considering that these two types of neurons are absolutely different in their properties and functions. Secondly, all the research conclusions were based on bioinformatic analysis of snRNA-Seq and scRNA-Seq data without any experimental validation. Since data of Mononle3 and SCENIC analysis are predictive, it is necessary to validate the expression dynamics of some critical genes such as SHANK3 and TCF4 that were mentioned in the manuscript. Furthermore, it is better to study their functions in OSNs and OE tissues. Here are my specific comments:

What is the difference between the e_iOSN and l_iOSN? Is it functional significance to classify iOSNs into these two subclusters since the G2M score of e_iOSN is not significantly higher than l_iOSN as shown in Figure 1h?

A pseudotime trajectory of OSN genesis was shown in Figure 2. However, this trajectory from GBC to mOSN has already been well-established. What are the new findings here? As to normalized fitted expression of trajDEG clusters and enriched GO analysis, authors should specify what genes represent in each trend cluster?

Figure S4 is missing in the supplementary figure file.

It is not clearly stated why the transcriptional similarities and difference between GBC/iOSN/mOSN and cortical neuron are studied? If other types of neurons are used, will it reach a similar conclusion as cortical neurons?

“Notably, the neuron pruning associated apoptotic process was specific to CENs, indicating less complex processes in OSNs than in CENs during the middle maturation stage.” I did not see the logics here. The neuron pruning means more complex process?

Line 207 “The pseudotime trajectories of GBCs, iOSNs, and mOSNs paralleled fetal, fetal to adolescence, and adolescence to adult CENs, respectively.” I am not sure if this parallel is appropriate. Authors should state the rationale.

To fully support the conclusion that OSNs could partially track the expression dynamics of ASD risk genes in CENs, the expression profile of representative genes should be verified between OSN and CEN development experimentally.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
Overall, the study performed by Song et al. has several strengths by leveraging biopsy to directly sample the OE from six living adult humans. Notably, this has been done with careful processing and quality control. The pseudotime analysis and subsequent integration with the Durante et al. dataset are thoughtfully executed. However, there are some revisions that could incorporated that would improve the overall manuscript.

Comments/Critiques

-Although understandable given the nature of the tissue input. There is a overall a small OSN sample size – only 381 cells and 891 OSNs after integrating with Durante et al. can result in lower power and stability.

-The OSN numbers could be increased, or one may need to add robustness checks i.e. bootstrap/subsample methods. Also, the authors should report the stability of key outputs and analysis. Please explicitly mention this beyond lines 291-292. Show whether any conclusions are changed (if any) with resampling.

-Moran’s I screen and dependence on UMAP neighborhoods. Moran’s I from monocle3 are standard and the spline fits is performed correctly, but can favor genes concentrated in the 2D UMAP neighborhoods. Please confirm with embedding-independent approach and report concordance i.e., the authors may want to fit GAM/nb-splines directly vs. pseudotime (Slingshot).

-Using Harmony globally and Seurat CCA for OSNs is reasonable but can result in over-correction, especially by dropping baches of < 30 OSNs and/or only one OSN subtype with only 6 PCs, 600 HVGs. This may remove variance, bias stage proportions, or under-represent signals due to the low PC/HVG settings. To address this, the analysis can be re-run without excluding OSN-batches to assess for bias. Also, please demonstrate that stage-order is preserved with more liberal HVG/PC settings. Finally, does the ordering remain the same when using different frameworks (scVI/scANVI)?

-SCENIC+ regulon analysis: While the authors ran the analysis 50x and kept targets recurring for 30x, < 1k OSNs in total

(and even fewer GBCs) can make the results unstable. Please quantify stability metrics across the runs and provide confidence ranks. It may also be worth performing orthogonal validation using with independent evidence e.g. developing-cortex regulon targets or subsample OSNs and GBCs separately with inference to look for persistence of signals parameters.

-As the authors mentioned, the developmental axis for cortical cells vs. OSNs are very different (decades vs. 3 months) and the discretization with Genes2Genes can force alignments. The authors may consider repeating with alternative bin counts and report alignment stability. It would also be useful to show results are stable across CEN subsampling.

Minor Comments
1. Figure 1i. BIP for bipolar should be changed to BPD (or figure legend should be modified to match the axis accordingly)
2. In Methods section (line 347 of main manuscript), were the same parameters used for the re-clustering of OSNs alone?
3. In line 235 of the manuscript, it is mentioned that SIX1 and PAX6 were both highly expressed in GBCs over pseudotime. However the trend only shows that SIX1. I would not consider PAX6 highly expressed since its expression level barely goes above 0.5. Maybe it's a typo in line 237.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)
This work is potentially very exciting, but includes fundamental problems in details at least in the present manuscript.

This study uses a very small number of subjects for snRNA-seq (only 6). This reviewer is aware that, due to an urgent medical need during the COVID pandemic era, some studies with OE biopsy were published with a small number of subjects. However, the neural epithelium in the nasal cavity exists only in a limited area, and the consistency of the biopsy that meets with the goal of the present study is uncertain. Furthermore, nasal cavity is highly influenced by environmental factors, whose impact may be even more robust than the impact by the genetic program. How do the authors control the influence of environmental factors on each individual? Smoking, living in urban or non-urban area (air pollution), infectious condition, potential nasal inflammation by these factors and so on. All these are to be well controlled. to address these, in addition to molecular study, the authors may want to conduct histological study (immunohistochemistry) for each sample. More detailed demographic summary that list the potential environmental factors at each individual level will be very important. Altogether, the authors need an extensive addition regarding the data of how such environmental factors and their impact on host responses are standardized for the present study. Furthermore, data from more subjects (at least 10: ideally more) will be needed for the analysis.

The analysis itself in the present study is great.

This reviewer likes the study goal that seeks for how much neurogenesis in the olfactory neural lineage may be a good model for brain neurogenesis. However, stating that "OSNs represent a potential proxy to study gene programs involved in neurogenesis in human brains" may not be correct in the use of [OSNs] in this context. OSNs are mature neurons, and the statement should be rephrased as "cells in the olfactory neuroepithelium represent .....

In all, I admit the potential of this project. The final goal that the authors aim is great. Nevertheless, at least at present, the fundamental problems exist. The central issue of how much each individual (even called as healthy subjects) is very different with each other under daily influence of environmental factors is missing in this manuscript. In the nasal cavity, environmental factors that happen to each individual in different manners may influence gene expression, which may be even more than the impact by the intrinsic genetic factors. Accordingly, the authors may be expected to conduct an extensive, long-term revision if they wish.

(Remarks on code availability)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have taken appropriate steps to address my previous comments. The revisions have improved the clarity of methods and results. Here are additional suggestions:

1. Cell-type relevance to genetic risk assessed using scDRS
-- It would be helpful to include prefrontal cortex neurons as a positive control, and fibroblasts and pericytes from the olfactory epithelium as negative controls, to better contextualize the scDRS findings.

-- I am also curious about the contribution of mOSN, e_iOSN, and l_iOSN cells from each participant. If these cell types are unevenly represented across subjects, inter-individual differences rather than cell-type–specific effects could underlie the observed genetic risk associations.

-- Another issue is that GBC and e_iOSN—the two cell types with the smallest sample sizes—showed significant enrichment. If these cells were derived from fewer subjects, whereas mOSN and l_iOSN were contributed by a larger and more diverse set of participants, greater inter-individual heterogeneity in mOSN and l_iOSN could potentially dilute the signal and contribute to the lack of significance.

2. Similarities and differences between OSNs and CENs.
-- The third and fourth paragraphs in this subsection are complex, and the results are difficult to interpret. Several definitions and cutoffs are unclear, and some conclusions may be overinterpreted. I suggest that the authors either clarify key details and temper some interpretations, or shorten these sections and move part of them to the supplementary materials.

-- Line 214: How was the “overlap between OSNs and CENs” defined?

-- Line 215: The statement “stronger convergence during early developmental stages” may be overstated. CENs were from which developmental stage?

-- Line 216: What is the justification for selecting layer 2/3 neurons as the reference?

-- Lines 218–222: How were gene sets such as shared-down, shared trans-up, and shared up-regulated defined?

-- Line 225: How were the “top-enriched biological processes” defined? What cutoff was used to determine “top”?

-- Line 226: Is this comparing enrichment results of down-trending genes in OSNs with enrichment results of all genes in CENs?

-- Lines 233–234: These statements appear to be overclaimed.

Minor comments:
-- It would be helpful if the authors could report the sample sizes of GBC and e_iOSN.

-- There are discrepancies between the tracked-changes manuscript and the clean version. For example, the tracked-changes version (line 535) has “The marker gene lists were obtained downloaded from Wang et al.’s data 34” whereas the clean version states “The marker gene lists were obtained from Wang et al.’s data 37”.

-- Fig. 4e, while the inclusion of an independent mouse dataset is valuable, why not also compare gene expression across GBC, e_iOSN, l_iOSN, and mOSN using the dataset analyzed in this study?

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
Authors almost solved all my concerns and here I recommended the acceptance of this manuscript for publication. One more suggestion is regarding my previous comment 2.7. Authors performed bulk RNA-Seq analysis on mature OSNs and GBCs at P2-P16 to validate the expression of ASD risk genes. While these experiment data were solid, it is better to show the expression changes at protein level.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
Although some limitations remain due to sample size, the authors did a good job responding to prior critiques. Supported by welcomed clarification, numerous additional analyses, and thoughtful responses to reviewer points, I think the study/manuscript is certainly of quality for publication in Nature Communications. I endorse acceptance.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)
The authors are very responsive to the questions and requests from this reviewer. Thus, this reviewer finds the present revision as successful in principle. Based on this progress, this reviewer will underscore the following two points and expect the authors to be continuously responsive.

First point:
In the original manuscript, the authors tended to highlight only OSNs in their statement. However, this reviewer provided a comment on this concern, to which the authors made a good revision (see below)

4.3, However, stating that "OSNs represent a potential proxy to study gene programs involved in neurogenesis in human brains" may not be correct in the use of [OSNs] in this context. OSNs are mature neurons, and the statement should be rephrased as "cells in the olfactory neuroepithelium represent .....
We agree with the reviewer that “OSNs” is not appropriate. We have revised our manuscript.
Revised main text
represent a potential proxy to study gene programs involved in
Overall, cells in the olfactory neuroepithelium neurogenesis in the human brain, providing an accessible model for investigating neurodevelopmental dysfunction in
psychiatric disorders.

Nevertheless, there remains inaccurate sentences because of limited highlight to OSNs in the revised manuscript. For example: “increasing evidence showed that the olfactory epithelium is directly affected (functional and molecular alterations in OSNs) in neuropsychiatric disorders16,17,24.” Including these studies, several groups have successfully used olfactory epithelium-derived neuronal cells in demonstrating the pathophysiology of the olfactory epithelium in neuropsychiatric conditions, but these may not be attributed only to OSNs. In many lab studies, indeed, olfactory epithelium-derived neuronal cells have been frequently used, but these may not be exactly OSNs. In short, the problem of the example above is resolved by removing the phrase of (……). The authors may want to revisit the entire manuscript to avoid similar mistakes.

Second point:
As far as this reviewer sees, at least one reviewer finds some concern in references. Indeed, several representative papers in this field seem to be missing. In particular, given that the merit of studying neuronal signatures from the olfactory epithelium rather than iPS cells (reprogramed cells) is the potential of disease-associated, development/aging-associated epigenetic signatures (F Gage and several others have highlighted the significance of non-reprogrammed neuronal cells in research), the past works related to epigenetic landscapes and development/aging-associated process are important. Accordingly, this reviewer recommends the authors to include:
- Mol Psychiatry. 2013 Jul;18(7):740-2. PMID: 22925834.
- Schizophrenia (Heidelb). 2025 Apr 23;11(1):68. PMID: 40268926.

Regarding the increasing evidence showing the olfactory epithelium is directly affected in neuropsychiatric disorders, the current three citations are appropriate. This reviewer recommends the authors to cite the following papers in this citation.
- Mol Psychiatry. 2024 May;29(5):1453-1464. PMID: 38321120, PMCID: PMC11189720
- Transl Psychiatry. 2018 Apr 18;8(1):81. PMID: 29666369.

(Remarks on code availability)

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have adequately addressed all the concerns I raised. I believe the manuscript is ready for publication.

(Remarks on code availability)
Their code appears clear and helpful, although I did not run it.

Reviewer #4

(Remarks to the Author)
The authors are responsive to reviewers' comments. I appreciate the potential of this manuscript.

Two remaining issues.

1) Although the authors stated the revision of the text with the additional references in their response letter, these changes are not reflected in the main text. This reviewer regards this as a mere mistake by the authors. However, such sloppy work is not appreciated. Please fix this problem.

2) In addition, a recent paper published in Nature Communications is to be mentioned to enhance the discussion regarding the utility of human olfactory epithelium in brain disorder research.
Olfactory cleft biopsy analysis of Alzheimer's disease pathobiology across disease stages.
D'Anniballe VM, Kim S, Finlay JB, Wang M, Ko T, Luo S, Whitson HE, Johnson KG, Goldstein BJ.
Nat Commun. 2026 Mar 18;17(1):2245. doi: 10.1038/s41467-026-70099-7.
PMID: 41851126

(Remarks on code availability)
I am not a data scientist, but a medical doctor. However, I believe that the editor has thoughtfully invited data scientists.

Reviewer #1 (Remarks to the Author):

The authors collected snRNA-seq data from the human olfactory epithelium (OE) of six living adult donors and integrated an independent dataset from four additional adults to assess the molecular profiles of olfactory sensory neurons (OSNs). Overall, the manuscript is well-written, presents a comprehensive analysis, and provides rich datasets of potential value to the field. The manuscript could be further improved by enhancing methodological clarity and rigor in data presentation. For example:

We thank the reviewer for the positive assessment of the manuscript and the helpful suggestions to improve clarity and rigor.

1.1
-- The method used to generate Figure 1i is not immediately clear, as it is described in the middle of the Methods section rather than aligned with the Results presentation. A direct citation to the relevant Methods subsection would be helpful.
-- The cutoff for statistical significance should be explicitly stated. A less stringent cutoff was used for this analysis.
-- The rationale for selecting particular GWAS summary statistics should be provided. For instance, the authors use unpublished schizophrenia (SZ) GWAS data with a smaller sample size, rather than the larger, published PGC SZ dataset (Nature, 2022). Justification for this choice is needed.

We have clarified the scDRS method details in the results section. The single-cell disease relevance score (scDRS) was evaluated based on the expression of disease-associated genes from genome-wide association studies (GWASs)[1].
We have changed the FDR cutoff from 0.1 to 0.05. (Fig. 1i)
We have used the GWAS summary statistics from the PGC SCZ dataset (Nature, 2022) to perform enrichment analysis.
Revised main text and figure:

> **Results**: The single-cell disease relevance score (scDRS) was evaluated based on the expression of disease-associated genes identified by genome-wide association studies (GWASs)[1].
> **Methods**: The GWAS summary files for disorders/traits of interest are: ADHD[2], ASD[3], BD[4], MDD[5], SCZ[6], AD[7], ALS[8], MS[9], PD[10], and stroke[11].

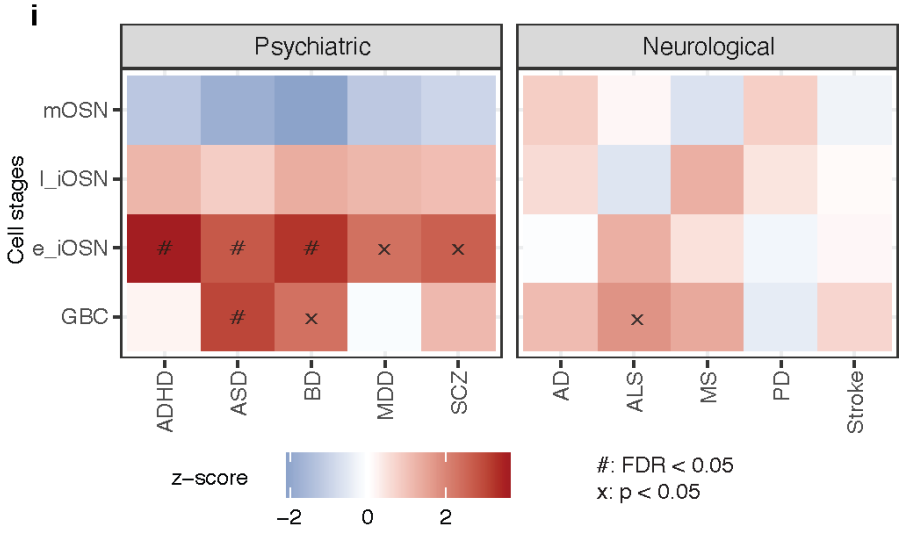


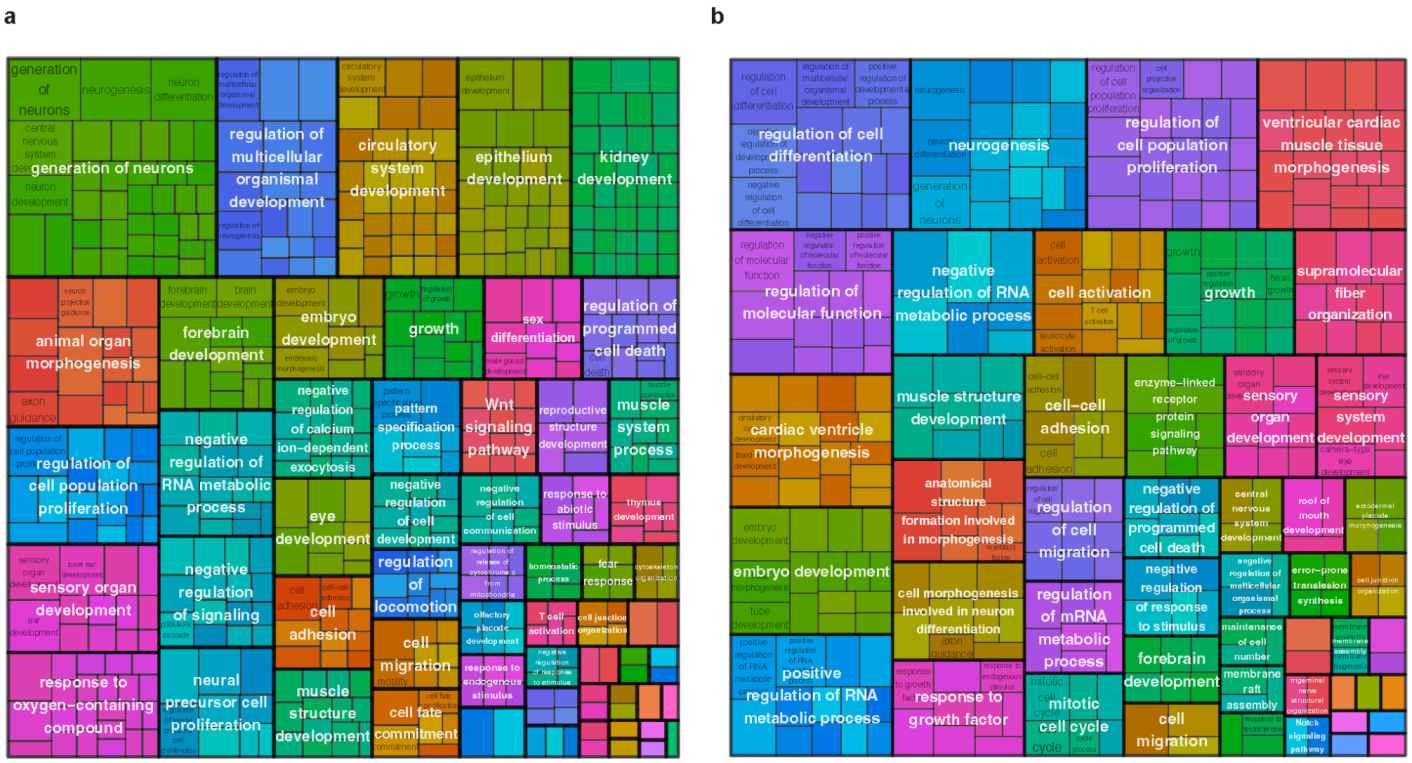
Fig. 1i. Single-cell disease relevance score (scDRS) of brain-related disease across the OSN lineage. '#' and 'x' represent FDR < 0.05 and p < 0.05, respectively.

1.2
-- The criteria for selecting enriched GO terms shown in the figure are unclear. It is understandable that not all significant terms can be shown in the main figure. However, according to Table S3, the subset presented in Fig. 2f does not correspond to the exact top three results. Clear selection criteria are needed to ensure objectivity and reproducibility.

We clarified the GO term selection criteria. Briefly, enriched GO terms were first subjected to redundancy reduction using rrvgo[12], and representative terms with the lowest adjusted p-values were selected from each semantic cluster. This procedure is now described in the figure legend and Methods, and full rrvgo outputs are provided in Supplementary Figures 7–9.

Revised Methods/supplementary figure:

> **Fig. 2f legend**: The top representative GO terms after redundancy reduction using rrvgo[12] are shown.
> **Methods**: The rrvgo (v1.14.2) package was applied to reduce the redundancy of GO lists by grouping similar terms based on their semantic similarity[12]. Terms with higher enrichment significance were picked to represent each group.



Supplementary Fig. 7: Function enrichment for down-trend genes. a, Enriched GO: BP terms of cluster1. Biological processes with similar semantics are grouped by rrvgo. The space used by the term is proportional to the significance. **b**, Enriched GO: BP terms of cluster2.

1.3

-- Figure 3a: The figure presents scaled correlation coefficients rather than raw Spearman values. Since raw correlation coefficients provide interpretable measures of effect size and strength, they should be shown to allow readers to assess the results more transparently.

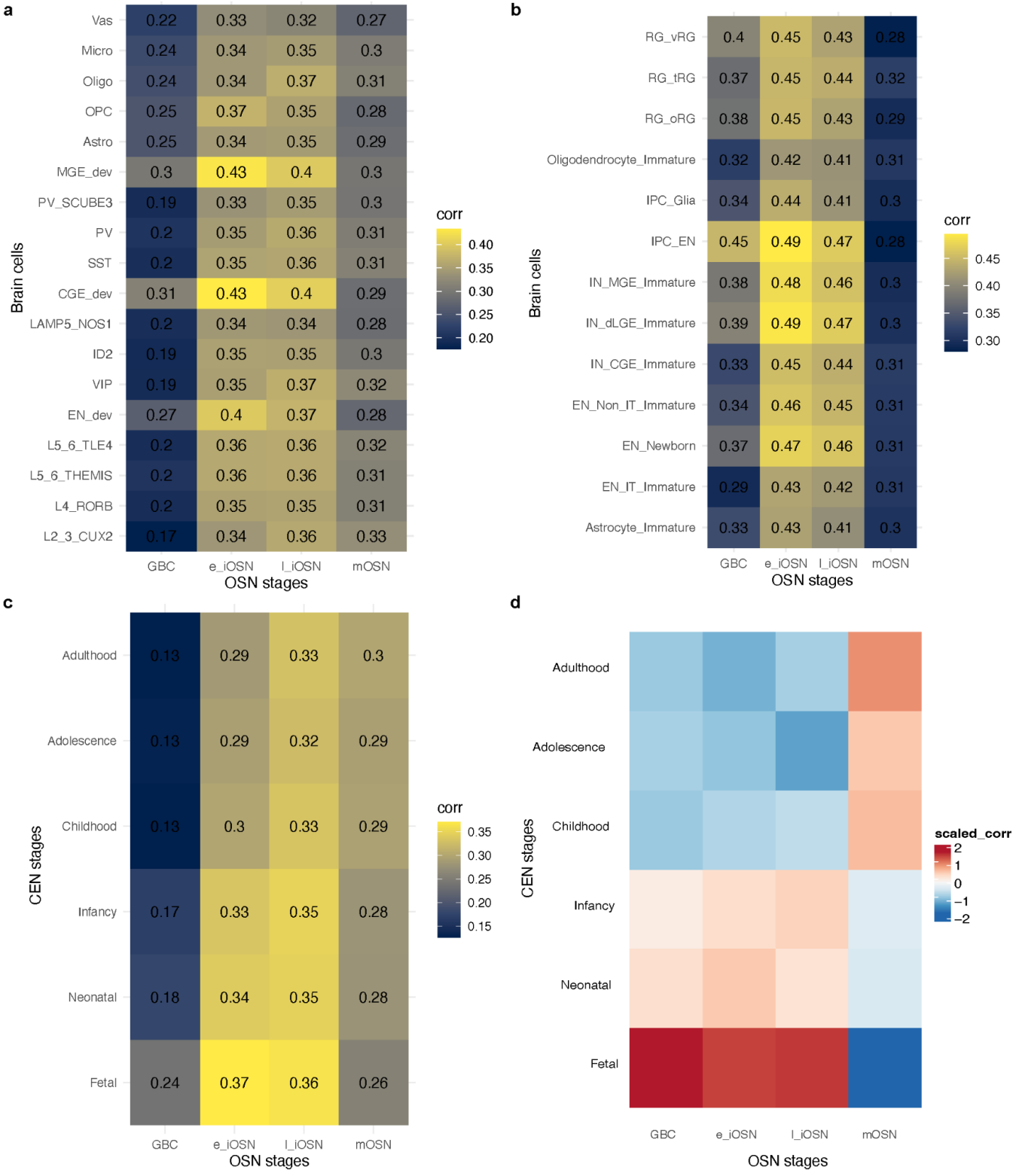
-- Figure 3b: It is unclear how the gene module score was calculated. Additional methodological detail should be provided.

We thank the reviewer for the suggestion. We have now provided the raw Spearman correlation coefficient (**Supplementary Fig. 10a**). We have also added the method for calculating the module score.

Revised main text and figure:

> **Methods**: To further validate the expressional resemblance, we also examined the module score of the top 50 marker genes for each brain cell type in OSNs, i.e, the average expression of a set of marker genes after subtraction by the average expression of a reference set of genes. The marker gene lists were obtained from Wang *et al's* data[13].

Results: Module scores were quantified for OSNs at different stages based on brain cell type–specific marker genes, representing the average expression of these markers in the OSN lineage relative to a random reference gene set.



Supplementary Fig. 10: Expression similarity between GBCs/OSNs and brain cell types. a-b, Spearman correlation of highly variable genes between GBCs/OSNs and cell types in the independent brain datasets from Herring *et al.* (a) and Wang *et al.*[13] (b). **c**, Correlation of highly variable genes between GBCs/OSNs and different stages of CENs. **d**, Correlation from panel (c) scaled by column. EN: excitatory neurons, IN: inhibitory interneurons, MGE: medial ganglionic eminence, CGE: caudal ganglionic eminence, dev: developing, Oligo: oligodendrocytes, OPC: oligodendrocyte precursor cells, RG: radial glia, IPC: intermediate progenitor cells, L2/3: layer 2/3.

1.4
Citing the following representative studies employing olfactory epithelium–derived cellular models for brain disorder research would be helpful in strengthening the motivation and conclusions of this work:

https://pubmed.ncbi.nlm.nih.gov/36291125/
https://pubmed.ncbi.nlm.nih.gov/36379214/
https://pubmed.ncbi.nlm.nih.gov/37250419/

We have incorporated the suggested references on olfactory epithelium–derived neuronal models into the Introduction to strengthen motivation and context. We agree that incorporating these references strengthens the motivation and conclusions of our work.

Revised main text:

> Olfactory sensory neurons (OSNs), located in the olfactory epithelium (OE) of the superior-medial nasal cavity[14], provide a unique opportunity to overcome these limitations, as they facilitate the investigation of neurodevelopmental processes that continue through adulthood. A distinctive feature of OSNs is their ability to regenerate throughout life via the proliferation and differentiation of globose basal cells (GBCs)[15]. Accordingly, OSNs have been utilized to unveil the neuronal signatures of psychiatric and neurological disorders such as schizophrenia, depression, Alzheimer's disease (AD), and Parkinson's disease[16,17]. For instance, dysregulation of Wnt signaling and microRNA-124-3p have been observed in schizophrenia patient-derived OSNs[18–22]. Similarly, gene expression changes associated with cognition were identified in an AD patient-derived OSN model[23]. Moreover, olfactory dysfunction, e.g., hyposmia, has also been reported in multiple neuropsychiatric disorders[24], indicating potential shared pathologic mechanisms between OSN deficits and brain disorders. Taken together, these observations suggest that the adult OSN lineage could serve as a model to investigate fundamental aspects of neuronal development, regeneration, and plasticity that are disrupted in neurodegenerative and neuropsychiatric disorders.

1.5
The codes are well-organized. It would be helpful if the input datasets corresponding to each script could be shared once the paper is accepted.

We have now provided the Seurat object for the integrated GBC/OSN cells, as well as source data corresponding to figures, and updated the code availability statement accordingly.

Reviewer #2 (Remarks to the Author):

In this manuscript, Song and colleagues compared human olfactory epithelium cells, including GBCs, iOSNs, mOSNs with cortical excitatory neurons based on the snRNA-Seq and reported scRNA-Seq datasets. They reported the convergence between OSNs and CENs, with shared and similar expression pattern genes related with psychiatric disorders and autism. Although authors showed some bioinformatic data, it is not fully well-grounded why the cortical neurons in the CNS and OSNs in the PNS were compared, considering that these two types of neurons are absolutely different in their properties and functions. Secondly, all the research conclusions were based on bioinformatic analysis of snRNA-Seq and scRNA-Seq data without any experimental validation. Since data of Mononle3 and SCENIC analysis are predictive, it is necessary to validate the expression dynamics of some critical genes such as SHANK3 and TCF4 that were mentioned in the manuscript. Furthermore, it is better to study their functions in OSNs and OE tissues. Here are my specific comments:

We thank the reviewer for raising important conceptual and validation-related concerns. We have clarified the rationale for the comparison between CEN and OSN and performed validation for critical genes using external data. Please find our point-by-point responses to the specific comments below.

2.1

What is the difference between the e_iOSN and l_iOSN? Is it functional significance to classify iOSNs into these two subclusters since the G2M score of e_iOSN is not significantly higher than l_iOSN as shown in Figure 1h?

Given iOSNs have lost the capacity for proliferation[25], the G2M score is expected to show no difference between them. The differences between early iOSNs and late iOSNs are distinguished by the expression of neuronal markers during different stages of differentiation and by olfactory receptor expression: Compared to l_iOSNs, e_iOSNs express higher levels of neural precursor markers (e.g., *SOX4*) (**Fig. 1g**). e_iOSN-specific markers were enriched in the mitotic cell cycle phase transition and regulation of developmental process (e.g., *LHX9*, *LHX2*, and *NFIB*), while l_iOSN-markers were associated with cell projection organization (e.g., *EFHD1* and *B3GNT2*) (**Supplementary Table 3**). Moreover, consistent with receptor transformations during olfactory neurogenesis[26], compared to e_iOSNs, l_iOSNs showed a gradual increase toward the singular olfactory receptor, consistent with receptor choice stabilization (**Supplementary Fig. 4b**). Further, e_iOSNs were more enriched for neuropsychiatric disorders (**Fig. 1i**) compared to l_iOSNs, supporting their distinction.

Revised main text and figure:

> iOSNs can be further divided into two subsets, early and late, based on the expression of neuronal markers during different stages of differentiation and on olfactory receptor expression[26](**Fig. 1g, Supplementary Fig. 4**). Compared to late iOSNs (l_iOSNs), early iOSNs (e_iOSNs) showed higher expression of neural precursor markers (e.g., *SOX4*) (**Fig. 1g**). e_iOSN-specific markers were enriched in the mitotic cell cycle phase transition and regulation of developmental process (e.g., *NFIB*, *LHX2*, and *LHX9*), while l_iOSN-specific markers were associated with cell projection organization (e.g., *EFHD1* and *B3GNT2*) (**Supplementary Table 2**). Moreover, consistent with receptor transformations during olfactory neurogenesis[26], compared to e_iOSNs, l_iOSNs showed a gradual increase toward a singular olfactory receptor (**Supplementary Fig. 4b**), indicating the stabilization of receptor choice.

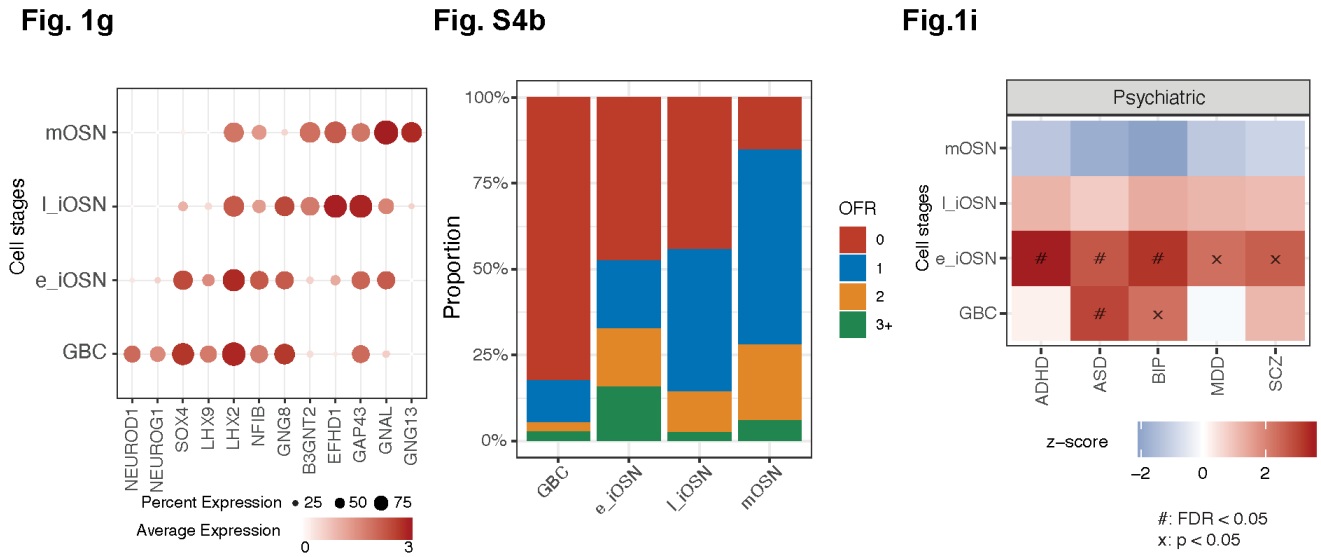


Figure: Comparison between early and late iOSNs. 1g, Expression level of key markers across OSN stages. Dot size and color represent the percentage of cells and the average expression level, respectively. **S4b,** Proportion of cells expressing varying numbers of olfactory receptor genes across cell stages. **1i**, Single-cell disease relevance score (scDRS) of neuropsychiatric disorders across the OSN lineages.

2.2

A pseudotime trajectory of OSN genesis was shown in Figure 2. However, this trajectory from GBC to mOSN has already been well-established. What are the new findings here? As to normalized fitted expression of trajDEG clusters and enriched GO analysis, authors should specify what genes represent in each trend cluster?

We thank the reviewer for the comment. While the general stages of OSN genesis have been established in model organisms, our trajectory, derived from snRNA-seq data on living donor biopsies, aligns with the established neurogenesis trajectory, validating its robustness. Furthermore, we provide a systematic characterization of the similarities and differences between OSNs and cortical excitatory neurons (CENs). Specifically, we first leveraged this trajectory to compare the developmental programs between OSNs and CENs. This reveals that early-stage functional convergence, with increasing divergence occurring during the middle and late stages of development and maturation. Genetic enrichment analysis revealed that GBC and iOSN were associated with neuropsychiatric disorders, further supporting their similarities. Second, we compared TFs between OSN and CEN lineage, which revealed that regulatory mechanisms are more conserved during earlier neurogenesis stages than later mature stages. Finally, we evaluated the trajectory alignment of risk genes for neuropsychiatric disease. We found that some ASD risk gene dynamics are matched between the two lineages, suggesting potential implications of the OSN lineage for studying the dysfunction of neurodevelopment. We have clarified these novelties in discussion.

Thanks for the suggestion on the genes represented in each trend. We have provided some representative genes in each trend cluster (**Fig. 2d**), such as *NEUROD1*, *SOX2*, *NOTCH2*, and *LHX2* in the down-trend cluster, *SLC7A1*, *BCL11B*, and *SLC7A5* in the trans-up-trend cluster, as well as *DRC1* and *FOXJ1* in the up-trend cluster.

Revised text and figure:

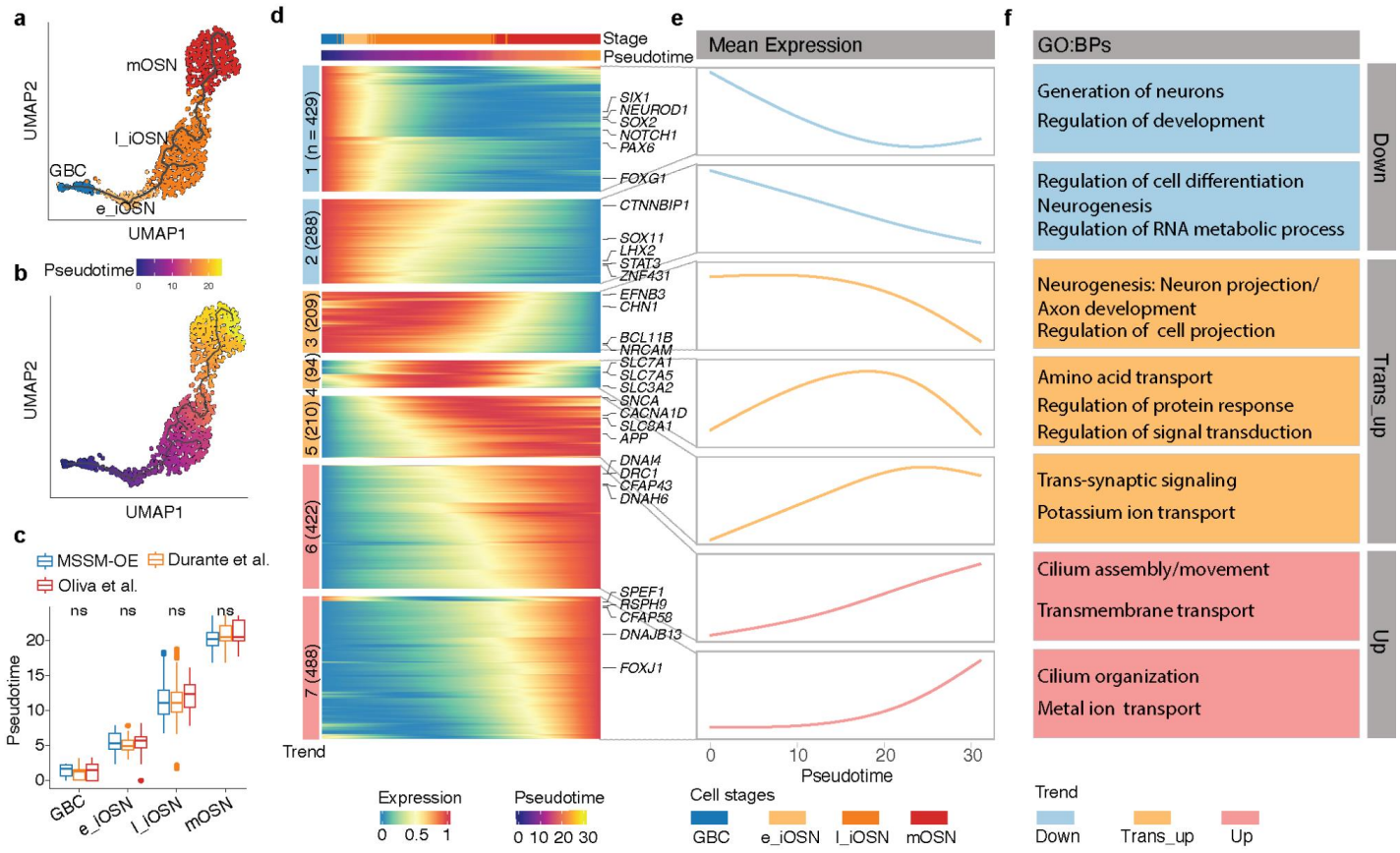


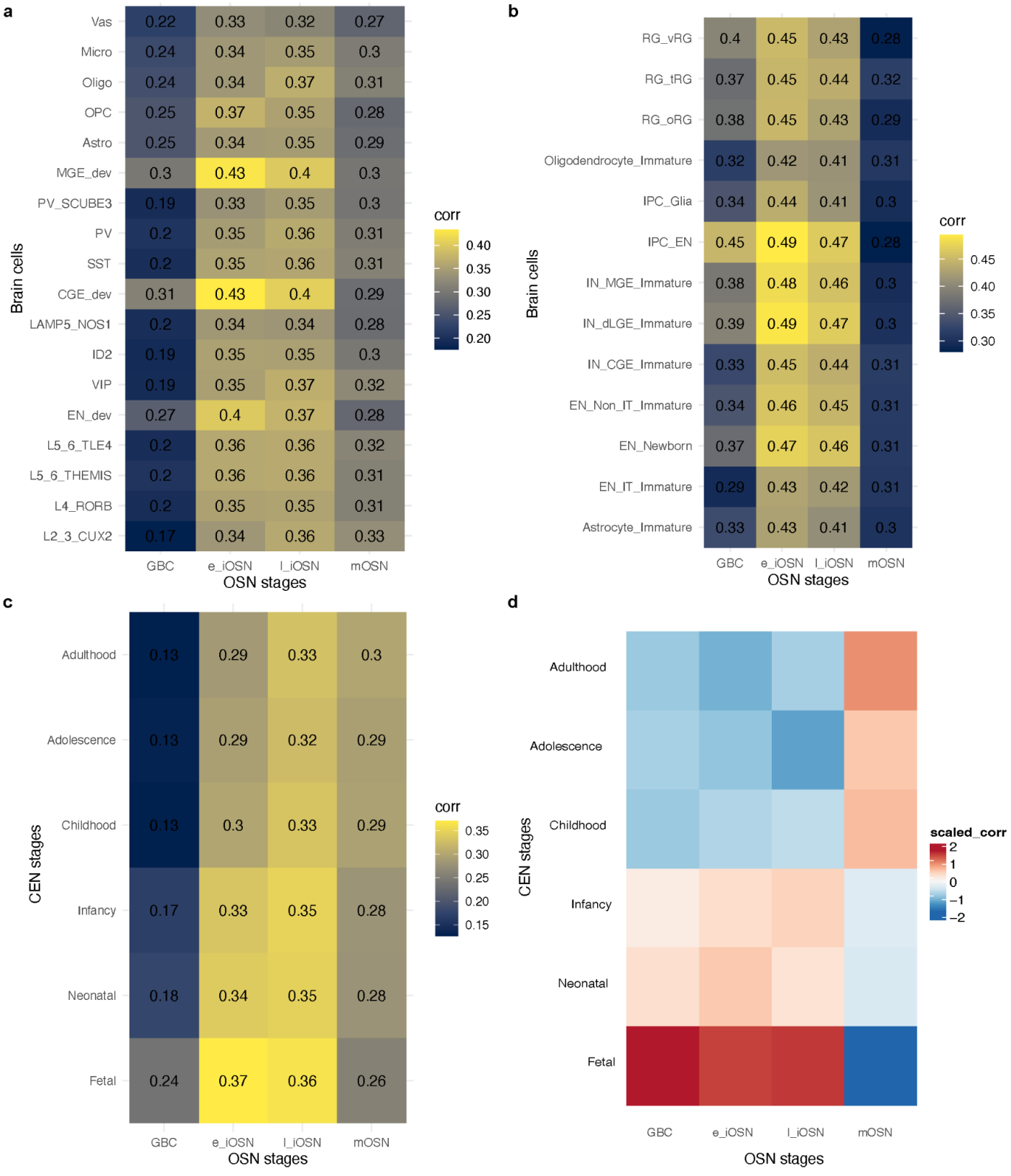
Fig. 2: Dynamics across OSN genesis and development. a-b, Developmental trajectory from GBCs to mature OSNs colored by cell stage **(a)** and pseudotime **(b)**. **c**, Distribution of pseudotime across cell types and data sources. The significance was determined using the Wilcoxon test. ns: not significant. **d**, Normalized fitted expression of trajDEG clusters along the pseudotime trajectory. The left color bar represents the dynamic trend patterns. The number in brackets represents the number of DEGs in each trend cluster. The top color bars denote the OSN stage and pseudotime. Selected representative genes are listed to the right. **e,** Mean expression for genes in each trend cluster along pseudotime. **f**, Enriched gene ontology biological processes (GO: BPs) for each gene cluster. The top representative GO terms after redundancy reduction using rrvgo[12] are shown.

Discussion: The molecular profiles of olfactory epithelium-derived progenitor neurons have been shown to resemble those of immature neurons in the human brain, clustering close to prenatal (mid-fetal) brain neurons[20,27]. However, their biological processes and regulatory mechanisms have never been systematically compared. Our comparative analysis of transcriptional and regulatory dynamics revealed both shared and distinct features between OSNs and CENs. We found that key developmental processes, including neurogenesis, cell proliferation, and transcriptional regulation, were conserved between GBCs/early iOSNs and neurons of the developing brain. Notably, TFs implicated in the cortical neuronal differentiation were also highly expressed in GBCs and early iOSNs. Furthermore, shared developmental processes, including axon development and synaptic signaling, were observed in OSNs and CENs during mid-to-late developmental stages. However, in contrast to the decades-long maturation period of brain neurons, OSNs exhibit a markedly shorter lifespan, typically lasting approximately three months[14]. In line with this, several CEN maturation-related processes, such as neuronal pruning and myelination, were absent in OSNs. Despite these differences, our findings underscore the potential of OSNs as a model system for investigating specific aspects of brain neurogenesis and development, particularly the utility of GBCs and early iOSNs in capturing early-stage neuronal processes.

Clinical phenotypes, such as smell deficits observed across multiple neuropsychiatric disorders, provide strong evidence for a connection between the olfactory system and neuropsychiatric disorders[28]. However, the extent of shared vulnerability and the underlying molecular mechanisms require further genetic support. Here, the polygenic enrichment of psychiatric disorders in GBCs and early iOSNs indicates higher expression of risk genes in early-stage OSNs, highlighting their potential to capture psychiatric-associated dysfunctions. The weaker association between OSNs and neurological and neurodegenerative disorders aligns with a primarily non-neuronal contribution to these conditions[29–32]. Moreover, GBC- and early iOSN-specific TF regulons were significantly enriched for psychiatric disorder risk variants, particularly those involved in CEN development. In addition, the matched expression dynamics of ASD risk genes between OSNs and CENs further reinforce this connection. Collectively, these results suggest that GBCs and early iOSNs represent promising cellular models for investigating the genetic underpinnings of neuropsychiatric disorders.

2.3 Figure S4 is missing in the supplementary figure file.

We thank the reviewer for the comment. We have provided **Supplementary Fig. 4** (now **Supplementary Fig. 10**).



Supplementary Fig. 10: Expression similarity between GBCs/OSNs and brain cell types. a-b, Spearman correlation of highly variable genes between GBCs/OSNs and cell types in the independent brain datasets from Herring *et al* (a) and Wang *et al*[13] (b). **c**, Correlation of highly variable genes between GBCs/OSNs and different stages of CENs. **d**, Correlation from panel (c) scaled by column. EN: excitatory neurons, IN: inhibitory interneurons, MGE: medial ganglionic eminence, CGE: caudal ganglionic eminence, dev: developing, Oligo: oligodendrocytes, OPC: oligodendrocyte precursor cells, RG: radial glia, IPC: intermediate progenitor cells, L2/3: layer 2/3.

2.4
It is not clearly stated why the transcriptional similarities and difference between GBC/iOSN/mOSN and cortical neuron are studied? If other types of neurons are used, will it reach a similar conclusion as cortical neurons?

We thank the reviewer for the insightful comment. We agree that our original text did not explain clearly enough why we focused on cortical excitatory neuron (CEN) development as the comparison reference. Importantly, we are not suggesting that OSNs recapitulate cortical neuron identity or function. Rather, we use CEN development as a well-characterized, brain-relevant developmental axis to ask a narrower question: to what extent do gene programs active during human OSN regeneration resemble gene programs during cortical neurogenesis and maturation, particularly those implicated in neuropsychiatric disease.

Our primary focus was to evaluate the potential of the OSN regeneration model to study cortical-related neurodevelopmental processes. Therefore, we did not compare OSN with other types of neurons. We have clarified the rationale for this comparison in the Introduction section. First, increasing evidence showed that the olfactory epithelium is directly affected (functional and molecular alterations in OSNs) in neuropsychiatric disorders[16,17,24]. This highlights that the olfactory system may capture disease-relevant molecular programs, providing a biologically meaningful rationale for comparison to central neurons. OSNs have been utilized to unveil the neuronal signatures of neurological and psychiatric disorders such as schizophrenia, depression, Alzheimer's disease, and Parkinson's disease[16,17]. Second, the unique regenerative ability throughout life makes the adult OSN a candidate model to investigate fundamental aspects of neuronal development, regeneration, and plasticity, which can be disrupted across various neurodegenerative and neuropsychiatric disorders. Third, we observed that the gene expression programs driving the transition from GBCs to mOSNs significantly overlapped with known CEN neurogenesis programs, and quantified the convergent (early) as well as divergent (mid to late maturation) stages. We also found a significant enrichment of neurodevelopmental and neuropsychiatric risk genes within the GBC and immature OSNs. This suggested a shared genetic association between the two systems.

We have revised the Introduction accordingly to make the rationale and scope of the comparison explicit.

Revised main text.

> Olfactory sensory neurons (OSNs), located in the olfactory epithelium (OE) of the superior-medial nasal cavity[14], provide a unique opportunity to overcome these limitations, as they facilitate the investigation of neurodevelopmental processes that continue through adulthood. A distinctive feature of OSNs is their ability to regenerate throughout life via the proliferation and differentiation of globose basal cells (GBCs)[15]. Accordingly, OSNs have been utilized to unveil the neuronal signatures of psychiatric and neurological disorders such as schizophrenia, depression, Alzheimer's disease (AD), and Parkinson's disease[16,17]. For instance, dysregulation of Wnt signaling and microRNA-124-3p have been observed in schizophrenia patient-derived OSNs[18–22]. Similarly, gene expression changes associated with cognition were identified in an AD patient-derived OSN model[23]. Moreover, olfactory dysfunction, e.g., hyposmia, has also been reported in multiple neuropsychiatric disorders[24], indicating potential shared pathologic mechanisms between OSN deficits and brain disorders. Taken together, these observations suggest that the adult OSN lineage could serve as a model to investigate fundamental aspects of neuronal development, regeneration, and plasticity that are disrupted in neurodegenerative and neuropsychiatric disorders.

2.5
"Notably, the neuron pruning associated apoptotic process was specific to CENs, indicating less complex processes in OSNs than in CENs during the middle maturation stage." I did not see the logics here. The neuron pruning means more complex process?

We agree that the presence of neuron pruning and apoptotic processes in CENs does not inherently justify the conclusion that OSN development is "less complex." To ensure scientific rigor and avoid over-interpretation, we have removed this statement from the revised manuscript. Instead, we now focus on the objective observation of specific developmental programs.

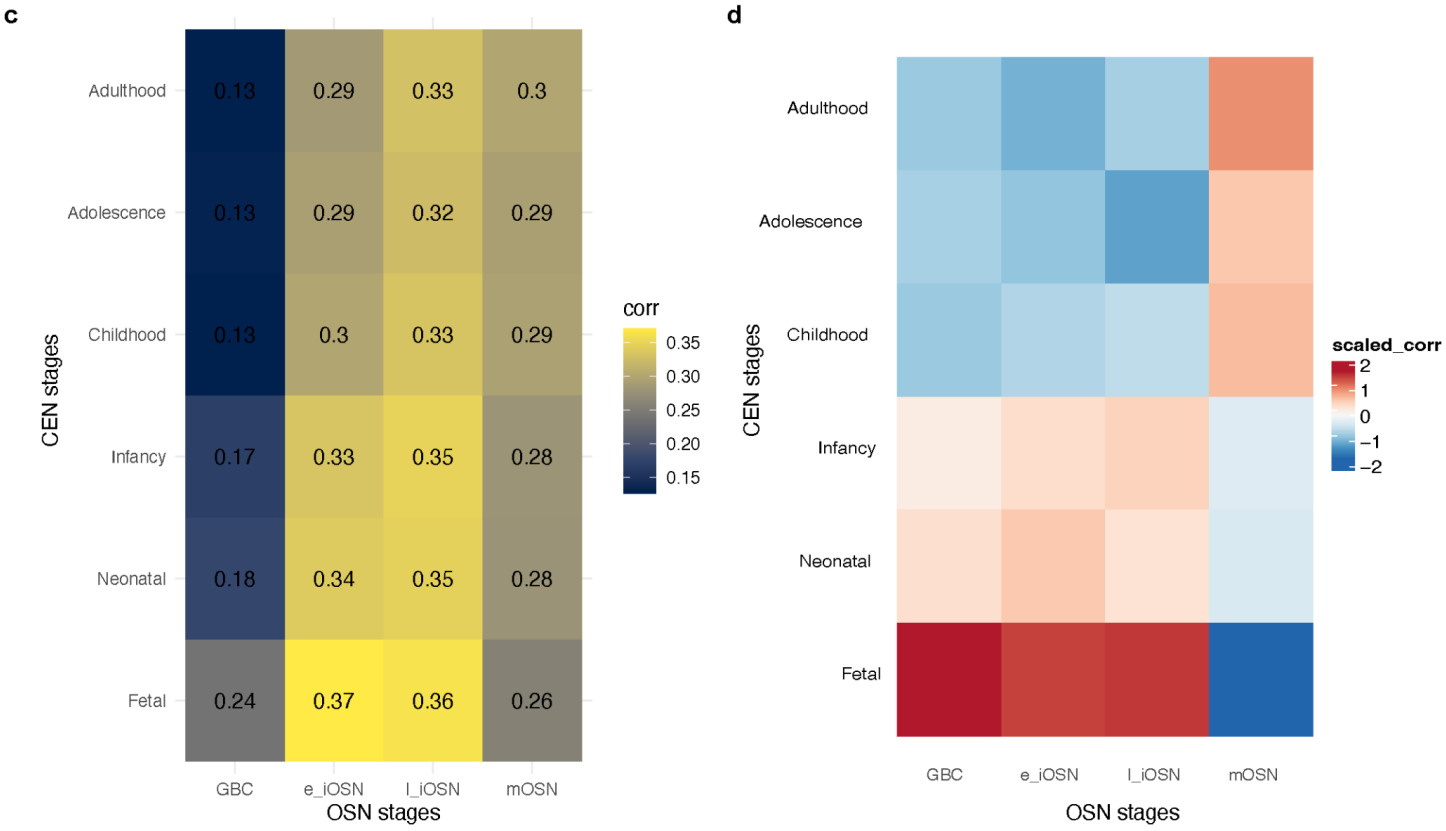
2.6

Line 207 “The pseudotime trajectories of GBCs, iOSNs, and mOSNs paralleled fetal, fetal to adolescence, and adolescence to adult CENs, respectively.” I am not sure if this parallel is appropriate. Authors should state the rationale.

We thank the reviewer for the comment. We agree with the reviewer that our original phrasing may have been interpreted as suggesting a direct temporal equivalence between OSN and CEN development. We have revised the text to clarify.

Revised main text.

> Concordant with the sequential transcriptomic correlation between OSN lineage and distinct developmental periods of CENs (**Supplementary Fig. 10c-d**), the pseudotime segments of GBCs, iOSNs, and mOSNs aligned with fetal, fetal-to-infancy, and childhood-to-adult CENs, respectively.



Supplementary Fig. 10: Expression similarity between GBCs/OSNs and Brain Cell Types. c, Correlation of highly variable genes between GBCs/OSNs and different stages of CENs. **d**, Correlation from panel (c) scaled by column.

2.7

To fully support the conclusion that OSNs could partially track the expression dynamics of ASD risk genes in CENs, the expression profile of representative genes should be verified between OSN and CEN development experimentally.

We agree with the reviewer that independent experimental verification would strengthen the conclusion that the OSN lineage partially tracks the expression dynamics of ASD risk genes observed in CENs. A systematic experimental assessment of ASD risk-gene dynamics in both developing cortex and olfactory lineage is beyond the scope of this study. To provide orthogonal evidence to the dynamics of these ASD risk genes, we analyzed an independent bulk RNA-seq dataset generated from cells isolated via FACS from the mouse olfactory epithelium during the critical developmental window (postnatal day P2 to P16)[33]. Specifically, mature OSNs were purified from Omp-GFP mice at postnatal stages P2, P7, and P16, while intermediate neuronal progenitors (GBC-INPs) were isolated from Neurog1-GFP mice at P3, P7, P9, and P16.

Validation against this dataset confirmed the concordance, with 75.5% (p = 0.0051) of down-regulated and 53.1% (p = 2.2e-4) of up-regulated ASD risk genes in CENs exhibiting the same trend in OSNs (**Supplementary Fig. 15**). We also provided examples of representative genes of each alignment pattern (**Fig. 4e-f**), including *SLC45A1*, *SHANK3*, and *SCN1A* for matched, medium-matched, and mismatched alignments. *SLC45A1*, a glucose transporter gene in the brain, which displayed a concordant up-trend in both the CEN and OSN lineages; *SHANK3*, coding a key postsynaptic density protein at glutamatergic synapses[34], exhibited a more modest increase in expression in OSNs than CENs. The OSN lineage mirrored the developmental up-trend seen in CENs, though the magnitude of the change was relatively lower. *SCN1A*, which encodes a sodium channel alpha subunit, exhibited specific high expression in mature CENs but not in OSNs.

We have incorporated this analysis into the revised manuscript (Results/Methods and new Supplementary Figure). We also noted the limitation of experimental validation in the Discussion.

Supplementary Fig. 15 **Fig. 4e** **Fig. 4f**

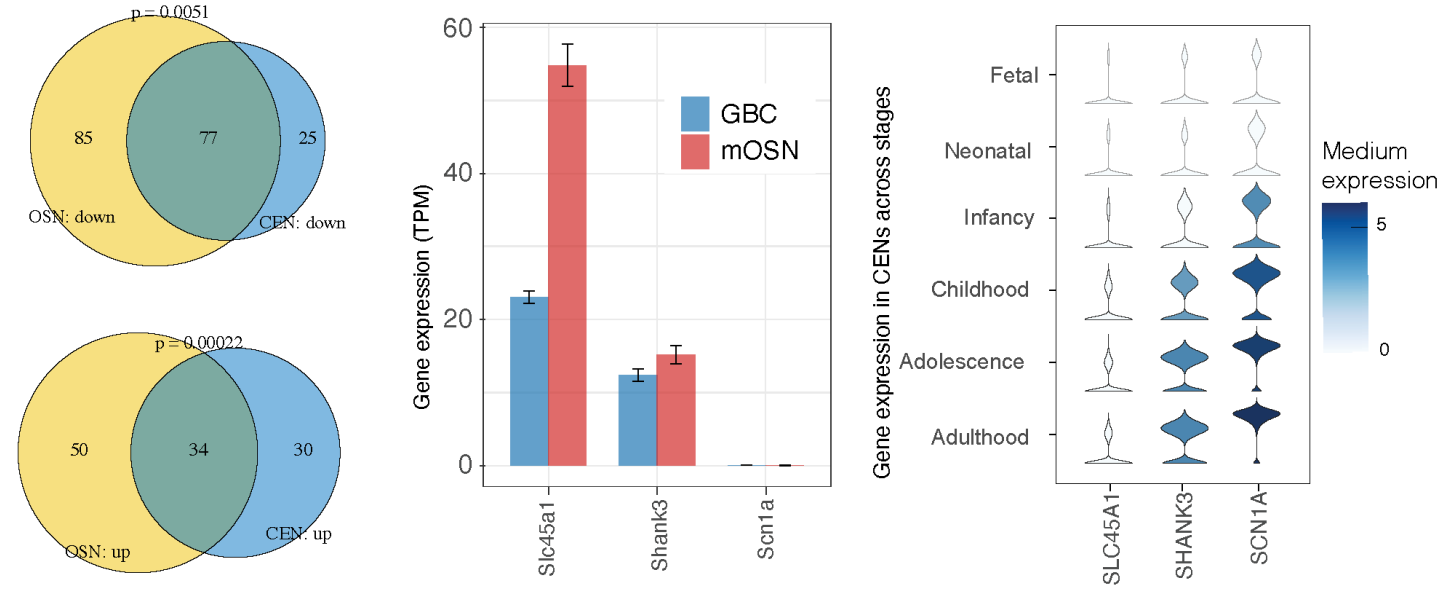


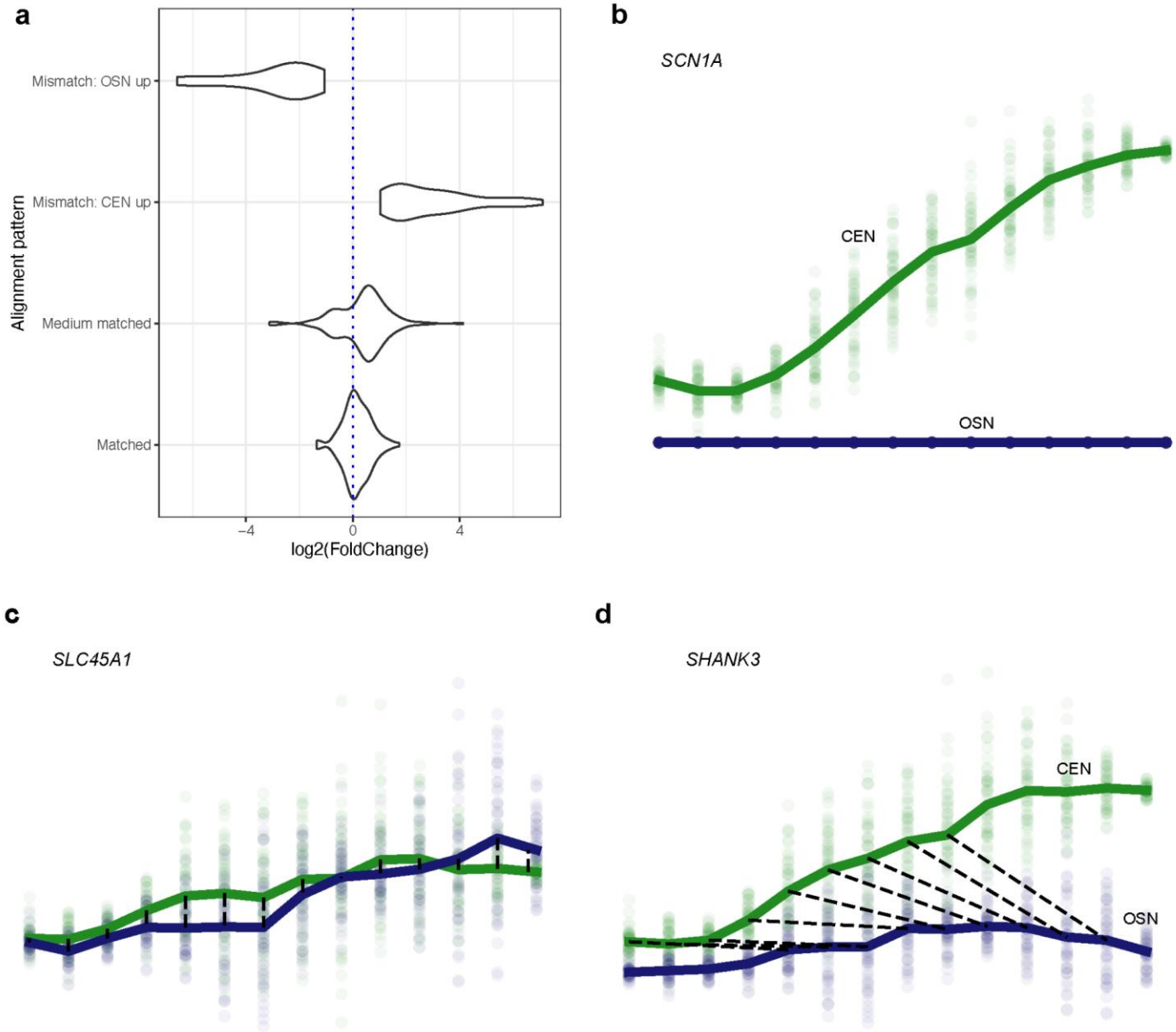
Figure: Concordance of the dynamic trend of ASD risk genes between CENs and mouse OSNs. Supplementary Fig. 15, The concordance of ASD risk genes between CENs and mouse OSNs. **Fig. 4e**, Expression of selected genes in fluorescence-activated cell sorting (FACS)-purified GBCs and mOSNs from an independent mouse dataset (GSE147460)[35]. **Fig. 4f**, Expression profiles of the selected genes across developmental stages of the CENs.

Revised main text, methods, and supplementary figures

> **Results**: Genes were categorized into three patterns based on their alignment similarity scores along the trajectory, including match (similarity > 0.75), medium match (0.25 - 0.75), and mismatch (< 0.25) (**Fig. 4c**). Given the bimodal distribution of fold changes in the mismatch cluster (**Supplementary Fig. 14a**), these genes were further subdivided into CEN-up and OSN-up groups. Functional enrichment analysis revealed that OSN-up and CEN-up mismatched genes were primarily associated with sensory organ morphogenesis and synaptic signaling, respectively (**Fig. 3d**). For instance, *SCN1A*, which encodes a sodium channel alpha subunit, exhibited specific high expression in mature CENs but not in OSNs (**Fig. 4e-f, Supplementary Fig. 14b**). Notably, out of 371 overlapping genes between ASD risk genes and expressed genes in OSNs and CENs, matched or medium-matched genes accounted for the larger proportion (**Fig. 4c**), highlighting that OSNs could partially track the expression dynamics of ASD risk genes in CENs. For example, *SLC45A1*, a glucose transporter gene in the brain, displayed a concordant up-trend in both the CEN and OSN lineages (**Fig. 4e-f, Supplementary Fig. 14c**). Similarly, *SHANK3*, encoding a key postsynaptic density protein at glutamatergic synapses[34], exhibited a more modest increase in expression in OSNs that partially mirrored the elevation observed in CENs (**Fig. 4e-f**, **Supplementary Fig. 14d**). Functional enrichment analysis for matched genes highlighted convergent biological processes (**Fig. 4c-d**), including transcription regulation, chromatin organization, and synaptic signaling. Validation against mouse

fluorescence-activated cell sorting (FACS) purified GBCs and OSN datasets confirmed this concordance, with 75.5% of down-regulated (p = 0.0051, Hypergeometric distribution) and 53.1% (p = 2.2e-4, Hypergeometric distribution) of up-regulated ASD risk genes in CENs exhibiting the same trend in OSNs (**Supplementary Fig. 15**). Collectively, these results support the utility of OSNs as a model system for studying neuronal dysfunction in neurodevelopmental disorders.

Discussion: In addition, although we validated the expression dynamics of some critical TFs and ASD risk genes using mouse RNA-seq data, further systematic experimental validation in human tissue contexts would strengthen the robustness of our findings. Finally, while the use of OSNs to identify dysfunctions in psychiatric disorders shows promise, further validation using specimens from donors diagnosed with these psychiatric conditions is needed. Case-control studies, alongside comparative analyses of brain tissues and organoid models, will be critical to establish the reliability and generalizability of OSN applications in neuropsychiatric disorder research.



Supplementary Fig. 14: The distribution of fold changes across each alignment pattern. a. Fold changes across alignment patterns. **b-d**. Expression dynamics of *SCN1A* ***(b)***, *SLA45A1* ***(c)***, and *SHANK3* ***(d)*** in OSNs and CENs along pseudotime. The dashed link represents the match status between OSN and CEN at each time point.

Reviewer #3 (Remarks to the Author):

Overall, the study performed by Song et al. has several strengths by leveraging biopsy to directly sample the OE from six living adult humans. Notably, this has been done with careful processing and quality control. The pseudotime analysis and subsequent integration with the Durante et al. dataset are thoughtfully executed. However, there are some revisions that could incorporated that would improve the overall manuscript.

We sincerely thank the reviewer for the thoughtful and detailed evaluation of our manuscript. We are pleased that the reviewer recognizes the strengths of our study. We fully agree that incorporating the suggested revisions will substantially improve the clarity and robustness of the manuscript. We have carefully addressed each point raised by the reviewer.

3.1
-Although understandable given the nature of the tissue input. There is a overall a small OSN sample size – only 381 cells and 891 OSNs after integrating with Durante et al. can result in lower power and stability.

We appreciate the concern regarding the potential impact of the limited sample size on statistical power and stability. To address this comprehensively, we have increased the sample size by integrating a new independent scRNA-seq dataset[36]. To control for the confounding effects of disease state, we strictly included only normal control samples and excluded samples derived from COVID-19 or presbyosmia patients. The total number of OSNs in the final analysis has been increased to 1,064 cells, which could increase the overall statistical power and stability of our findings.

Revised main text:

> To strengthen the robustness of our findings, we integrated our dataset with two normosmic control cohorts from Durante *et al.*[37] and Oliva *et al.*[36] (**Fig. 1e-f**). Within the combined dataset (n = 1,064 individual GBC and OSN profiles), cells were predominantly clustered by developmental stage rather than data source.

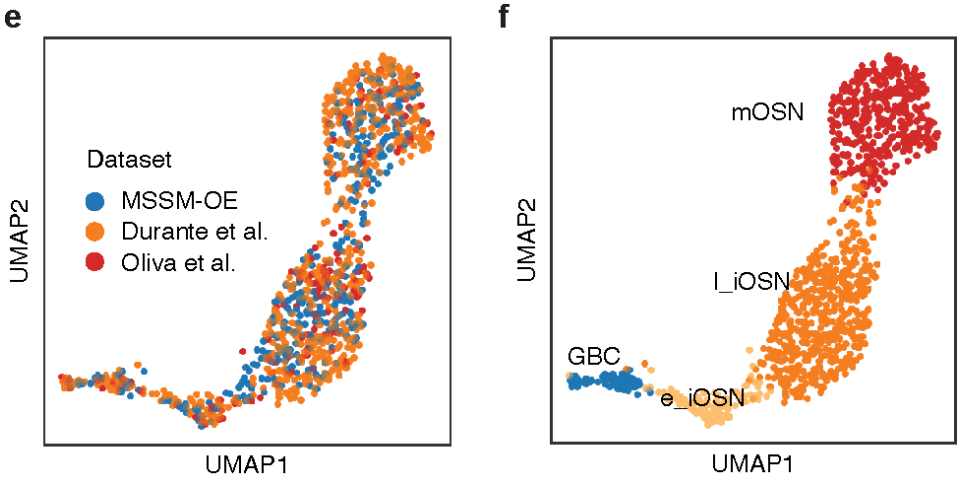
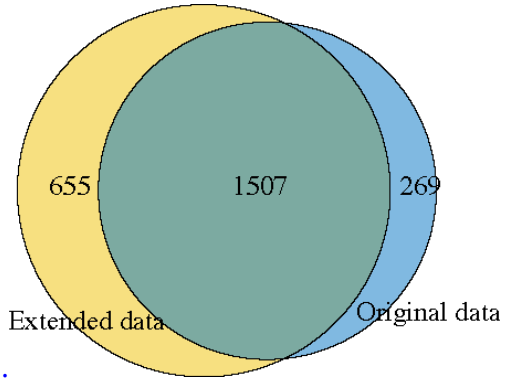


Fig. 1e-f, UMAP visualizations of integrated GBCs and OSNs from the current study (MSSM-OE) and Durante *et al.*'s dataset[37] and Oliva *et al.*'s dataset [36], with nuclei colored by data source (1e) and cell stage (1f).

3.2
-The OSN numbers could be increased, or one may need to add robustness checks i.e. bootstrap/subsample methods. Also, the authors should report the stability of key outputs and analysis. Please explicitly mention this beyond lines 291-292. Show whether any conclusions are changed (if any) with resampling.

We thank the reviewer for the constructive suggestion regarding sample size and the need for robustness validation. We have not only expanded our dataset but also performed comprehensive stability checks to ensure our conclusions are not biased by sample size. We have integrated a new independent cohort[36], increasing the total OSN lineage count to 1,064 cells (**Fig. 1e-f**). We observed a highly significant overlap in differentially expressed genes along pseudotime (trajDEGs) between the original (MSSM-OE & Durante et al.'s) and expanded datasets (MSSM-OE, Durante et al's dataset, and Oliva et al.'s dataset) (Hypergeometric test, $p < 2.2e-16$, **Fig. R1**), indicating that the core transcriptional programs are consistently captured.

As suggested, we have performed several robustness checks, including subsampling and methodological cross-validation for trajectory inference, trajectory alignment between GBCs and CENs, and gene regulatory networks. The details can be seen below. Notably, none of our previous conclusions were altered after increasing the sample size or performing resampling. The core findings—specifically the OSN developmental trajectory and its capacity to track specific CEN gene dynamics—remained highly stable across all validation tests. We have added a detailed description of these stability analyses to the revised manuscript (see below)



.

Figure R1: Overlap between trajDEGs identified from the original (MSSM-OE & Durante et al's) and expanded datasets (MSSM-OE, Durante et al's dataset, and Oliva et al.'s dataset)

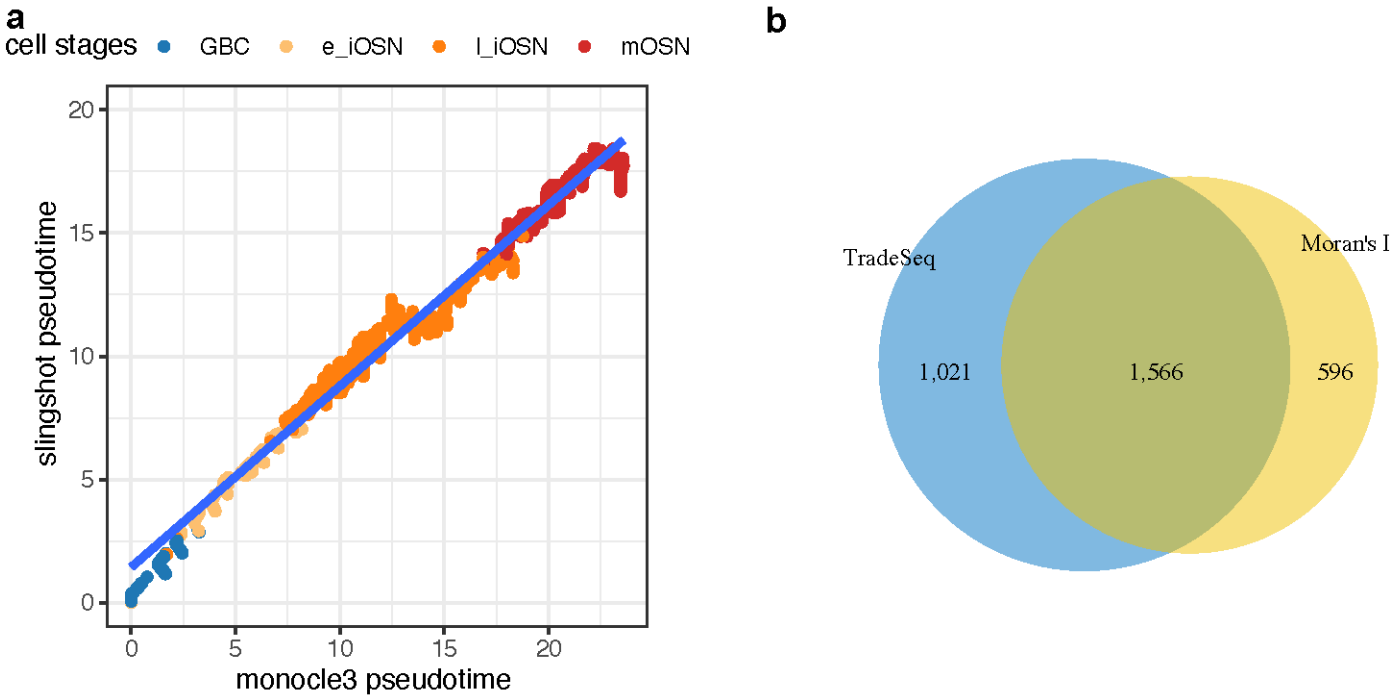
3.3

-Moran's I screen and dependence on UMAP neighborhoods. Moran's I from monocle3 are standard and the spline fits is performed correctly, but can favor genes concentrated in the 2D UMAP neighborhoods. Please confirm with embedding-independent approach and report concordance i.e., the authors may want to fit GAM/nb-splines directly vs. pseudotime (Slingshot).

We thank the reviewer for this insightful suggestion. We re-inferred the developmental trajectory using Slingshot [38] and identified DEGs along this pseudotime using tradeSeq[39], which fits a general additive model (GAM) directly against the Slingshot pseudotime. The pseudotime values generated by our original Monocle3 analysis and the new Slingshot analysis were exceptionally highly correlated (Spearman's rho = 0.99, p < 2.2e-16, **Supplementary Figure 5a**). We observed a significant overlap between the DEGs identified by Moran's I and those identified by tradeSeq (Hypergeometric test, p < 2.2e-16; Jaccard Index = 0.492, **Supplementary Figure 5b**). These results confirm that the trajDEGs we reported are biologically driven and consistently captured across different statistical frameworks, regardless of the 2D UMAP neighborhood structure. We have added this analysis to the revised manuscript.

Revised main text and figures:

> Moreover, we cross-validated our findings using Slingshot[38] and tradeSeq[39]. The high correlation of pseudotime (R = 0.99, p < 2.2e-16, Spearman correlation, **Supplementary Figure 5a**) and the significant overlap of trajDEGs (hypergeometric distribution, p < 2.2e-16; Jaccard index = 0.492, **Supplementary Fig. 5b**) confirmed the stability of our inference.

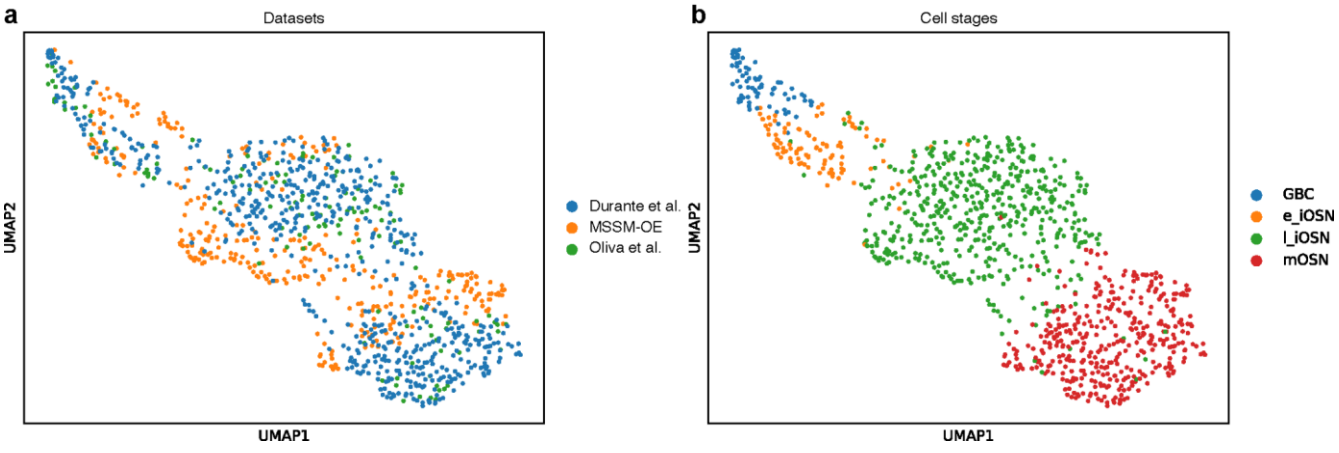


Supplementary Fig. 5: Pseudotime trajectory and differentially expressed genes along the trajectory (trajDEGs). a, Correlation of pseudotime assigned by the Slingshot and the Monocle3. **b**, Overlap between trajDEGs identified by fitting a Generalized Additive Model (GAM) using the tradeSeq against slingshot pseudotime and those identified using Moran's I against monocle3 pseudotime.

3.4

-Using Harmony globally and Seurat CCA for OSNs is reasonable but can result in over-correction, especially by dropping batches of < 30 OSNs and/or only one OSN subtype with only 6 PCs, 600 HVGs. This may remove variance, bias stage proportions, or under-represent signals due to the low PC/HVG settings. To address this, the analysis can be re-run without excluding OSN-batches to assess for bias. Also, please demonstrate that stage-order is preserved with more liberal HVG/PC settings. Finally, does the ordering remain the same when using different frameworks (scVI/scANVI)?

We thank the reviewer for the constructive comments. In the revised version, we did not drop batches with a low number of cells. We integrated datasets based on the top 30 PCs, the top 3000 HVGs. The stage order is preserved compared with 6 PCs and 600 HVGs. We also applied scvi to integrate these three datasets[40] (**Supplementary Fig. 3**), and the order remains the same. Within the combined dataset (n = 1,064 individual GBC and OSN profiles), cells were predominantly clustered by developmental stage rather than data source. While subtle batch effects were observed between data sources, likely arising from different sequencing methodologies (snRNA-seq in MSSM-OE cohort vs. scRNA-seq in Durante *et al.*'s and Oliva *et al.*'s cohort), the consistent cell orders confirmed the robustness of the integration. To further control the batch effects, the covariates, including the data source, sex, age, and read count, were regressed out.



Supplementary Figure 3: UMAP visualizations of integrated GBCs and OSNs across cohorts. a-b, GBCs and OSNs from the current study (MSSM-OE), Durante *et al*'s cohort[37], and Oliva *et al*'s cohorts[36] were integrated using scVI and visualized via UMAP. Nuclei are colored by **(a)** data source and **(b)** developmental cell stage assigned by Seurat CCA integration.

Revised main text and figures:

> **Results**: Within the combined dataset (n = 1,064 individual GBC and OSN profiles), cells were predominantly clustered by developmental stage rather than data source. While subtle batch effects were observed between data sources, likely arising from different sequencing methodologies (snRNA-seq in MSSM-OE cohort vs. scRNA-seq in Durante *et al*.'s and Oliva *et al*.'s cohort, **Supplementary Fig. 3**), the consistent cell orders confirmed the robustness of the integration.
>
> **Methods**: After removing low-quality cells, a total of 1,064 cells were retained. The top 30 PCs, the top 3,000 HVGs, and 30 nearest neighbors were selected to integrate data and co-embed shared cell types across datasets and batches. To ensure the robustness of our cell taxonomy, we independently validated the integration using scVI[40]. Data source and donor identity were treated as batch variables during model training.

3.5

SCENIC+ regulon analysis: While the authors ran the analysis 50x and kept targets recurring for 30x, < 1k OSNs in total (and even fewer GBCs) can make the results unstable. Please quantify stability metrics across the runs and provide confidence ranks. It may also be worth performing orthogonal validation using with independent evidence e.g. developing-cortex regulon targets or subsample OSNs and GBCs separately with inference to look for persistence of signals parameters.

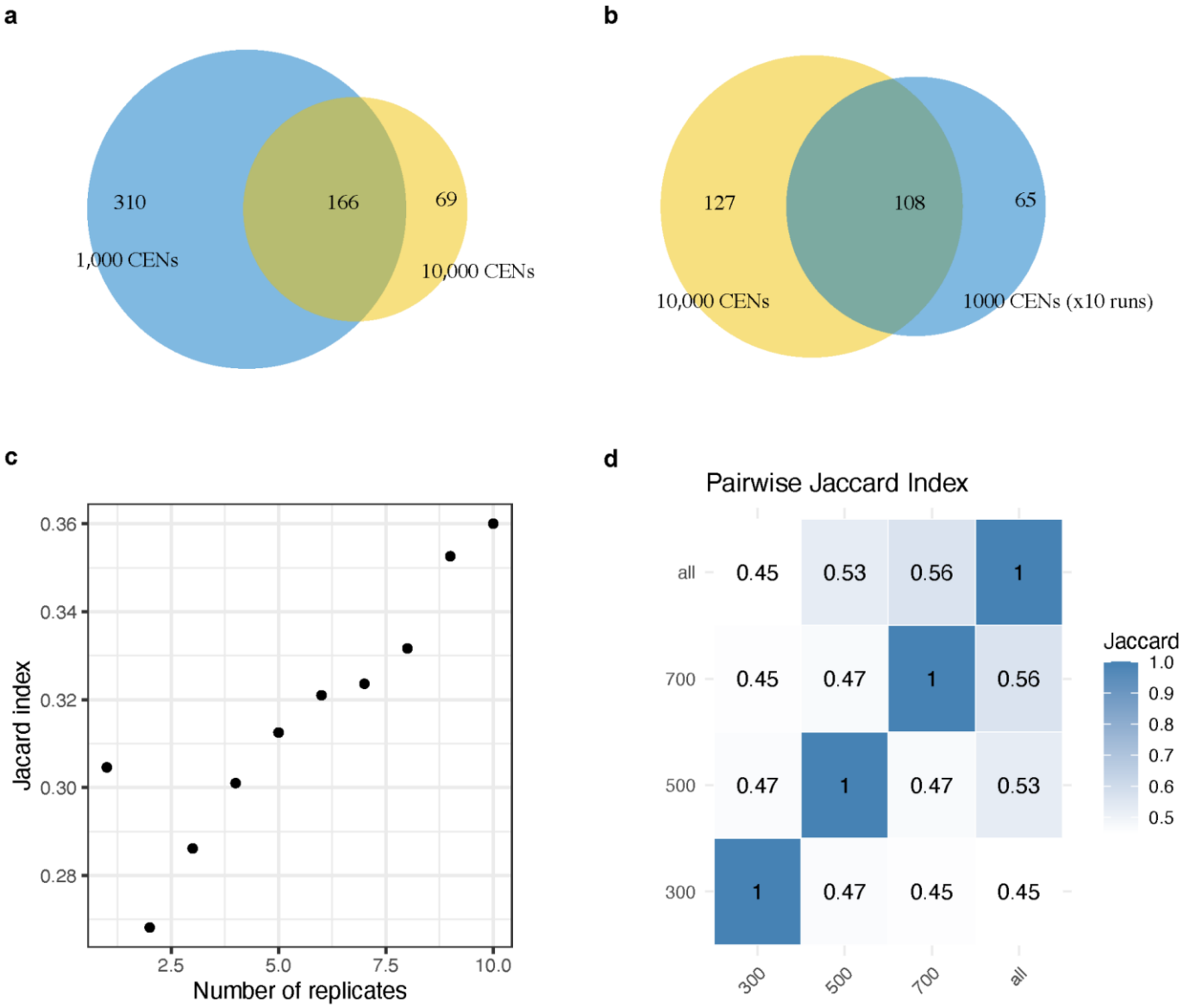
We thank the reviewer for raising this point. We agree that GRN inference can be sensitive when cell numbers are limited, particularly for rarer populations such as GBCs. To address this, we added explicit stability metrics across runs and performed orthogonal and subsampling-based validations.

We now report, for each TF regulon, its occurrence frequency across 50 independent SCENIC runs (**Fig. 5b / Supplementary Fig. 16**). We use this frequency as an empirical confidence measure and rank regulons accordingly. In the revised analysis, we also apply a more stringent consistency filter (retaining regulons detected in ≥40/50 runs) to focus downstream analyses on the most reproducible signals.

As an external benchmark, we ran the same pipeline on a developing cortical excitatory neuron (CEN) dataset and compared regulons inferred from 1,000 vs 10,000 cells. Regulon sets inferred from 1,000 cells showed lower concordance with the 10,000-cell reference (Jaccard = 0.30; **Supplementary Fig. 16a**), consistent with increased variability at smaller sample sizes. Importantly, applying a multi-run consistency filter improved concordance (**Supplementary Fig. 16c**), and the "most robust" regulons from the 1,000-cell setting (those consistently recovered across all 10/10 runs) showed improved agreement with the 10,000-cell results (**Supplementary Fig. 16b**). These analyses support our use of multi-run consensus filtering as a practical way to reduce run-to-run variability.

We additionally downsampled our combined GBC/OSN dataset to 300, 500, and 700 cells and re-inferred regulons. Compared with the full dataset, regulon overlap remained moderate (pairwise Jaccard 0.45–0.56; **Supplementary Fig. 16d**), indicating that the dominant regulon signals are reproducible under subsampling.

In addition, SCENIC is known for its robustness for sparse low-cell count experiments. In the SCENIC paper[41], the authors found SCENIC is robust with as few as 100 random single-cell transcriptomics.



Supplementary Fig.16: TF regulon identification across distinct cell counts. a, Overlap of TF regulons identified from 1,000 CENs and 10,000 CENs. **b**, Overlap between TF regulons identified from the 10,000 CENs and the most robust regulons from 1,000 CENs (those consistently identified in 10 out of 10 independent multi-runs). **c**, The Jaccard index of the TF regulons identified from 10,000 CENs and from 1,000 CENs with various replicates out of 10 runs. **d**, Pairwise Jaccard index of the TF regulons identified from varying subsampling scales of GBCs and OSNs.

Revised main text and figures:

Main text: Because low cell numbers increase the risk of spurious signals (**Supplementary Fig. 16)**, for downstream analysis, we prioritized highly confident regulons by retaining only TF regulons that were consistently detected in at least 40 of 50 independent runs (**Fig. 5b**, **Supplementary Fig. 16**).

Methods: To assess the robustness of the GRN network derived from a limited sample size, we performed a validation using an independent CEN dataset. We compared TF regulons identified from a subsampled CEN dataset (~ 1,000 CEN cells) against those derived from a well-powered dataset (10,000 CEN cells). Furthermore, we ran pySCENIC 10 times to confirm the advantage of the multi-run strategy in mitigating false positive regulon calls. To assess the robustness of our OSN findings, we performed a downsampling analysis (to 300, 500, and 700) and compared the resulting regulons with those identified from the full dataset.

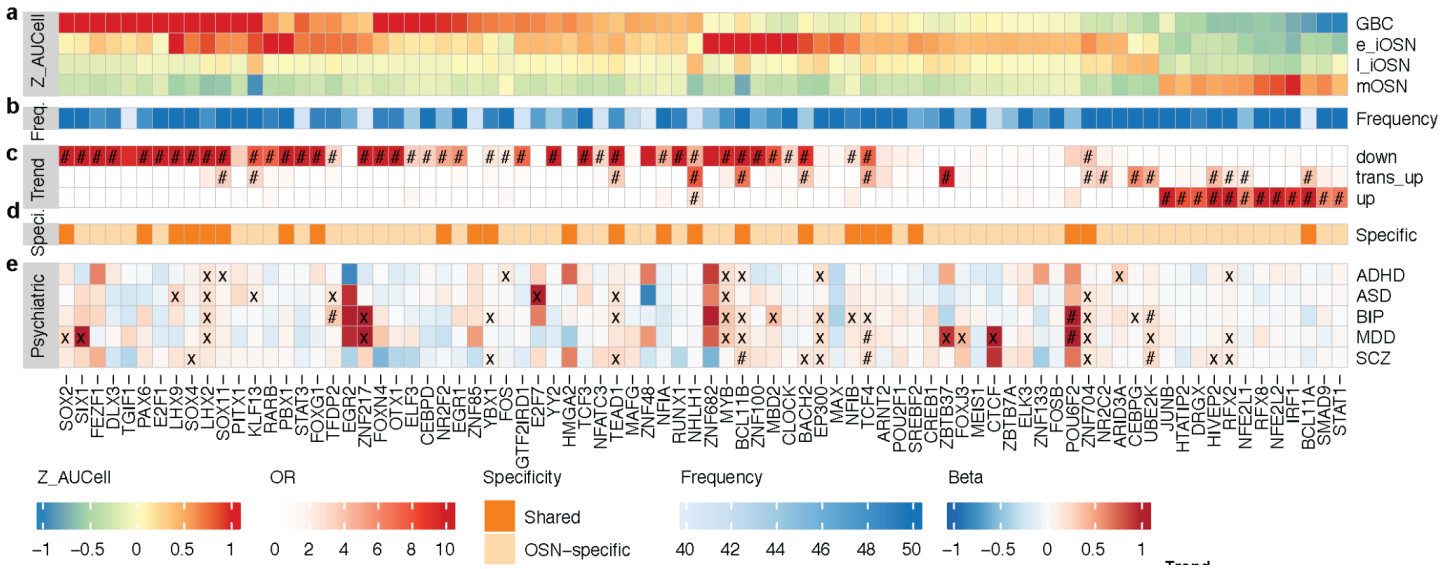


Fig. 5: Association of OSN stage-specific TFs with neuropsychiatric disorders. a, z-scaled AUCell scores of TF regulon activity across OSN stages. **b**, The frequency of occurrence for each TF regulon across the 50 independent multi-runs. **c,** Enrichment of TF regulons in trajDEG trends. The color indicates the odds ratio (OR, Fisher's exact test). '#' represents FDR < 0.05. **d,** Color bar categorizing TFs as OSN-specific or shared between OSNs and CENs. **e**, MAGMA enrichment illustrating the association of psychiatric disorders with TF regulons. 'x' and '#' indicate p < 0.05 and FDR < 0.05, respectively.

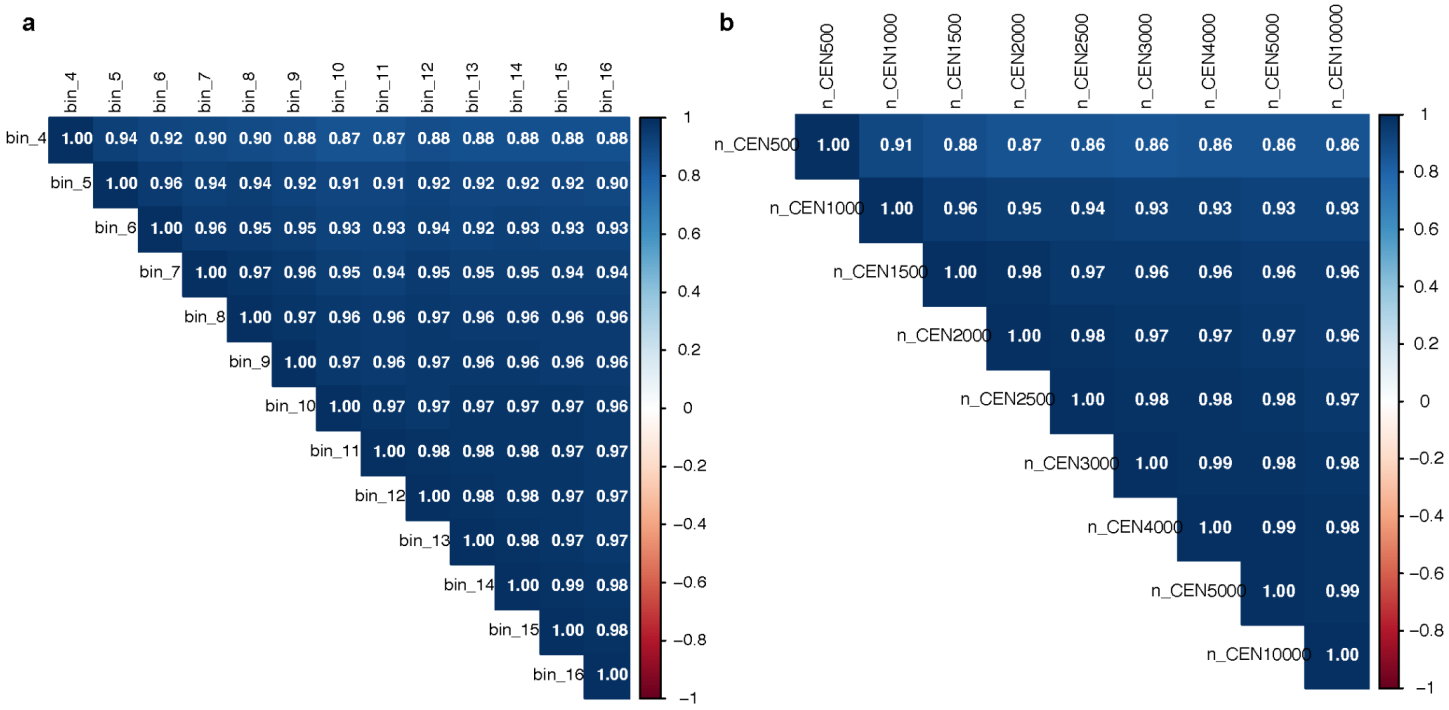
3.6

As the authors mentioned, the developmental axis for cortical cells vs. OSNs are very different (decades vs. 3 months) and the discretization with Genes2Genes can force alignments. The authors may consider repeating with alternative bin counts and report alignment stability. It would also be useful to show results are stable across CEN subsampling.

We thank the reviewer for the suggestion. To systematically assess the robustness of the gene alignment results, we evaluated the similarity scores under varying parameter conditions: (1) Stability across bin counts: We repeated the alignment process using a wide range of pseudotime bin counts (from 4 to 16). The alignment similarity scores exhibited a remarkably significant correlation between pairwise bin counts (**Supplementary Fig. 13**). (2)Stability across CEN subsampling: We performed random subsampling of the CEN dataset, varying the cell count from 500 to 10,000 cells. Despite the significant variation in sample size, the alignment similarity scores showed high correlation with each other (**Supplementary Fig. 13**). The high correlation between similarities from these varied conditions confirmed the reliability and stability of the alignment. We have added this analysis to the revised manuscript.

Revised main text and figures:

> The gene-level alignment similarity was quantified based on the state concordance across pseudotime bins. To validate the robustness of this measure, we examined the impact of varying key parameters, including the number of pseudotime bins (n=4 to 16) and dataset size (number of CENs = 500 to 10,000) (**Supplementary Fig. 13**). The high correlation between similarities from these varied conditions confirmed the reliability and stability of the alignment.



Supplementary Fig. 13: Correlation of alignment similarity percentage. (a) Correlation of alignment similarity percentage derived from varying pseudotime bin counts. **(b)** Correlation of alignment similarity percentages obtained by subsampling the CEN dataset to different cell counts.

Minor Comments

1. Figure 1i. BIP for bipolar should be changed to BPD (or figure legend should be modified to match the axis accordingly)

We have revised the legend to keep it consistent.

2. In Methods section (line 347 of main manuscript), were the same parameters used for the re-clustering of OSNs alone?

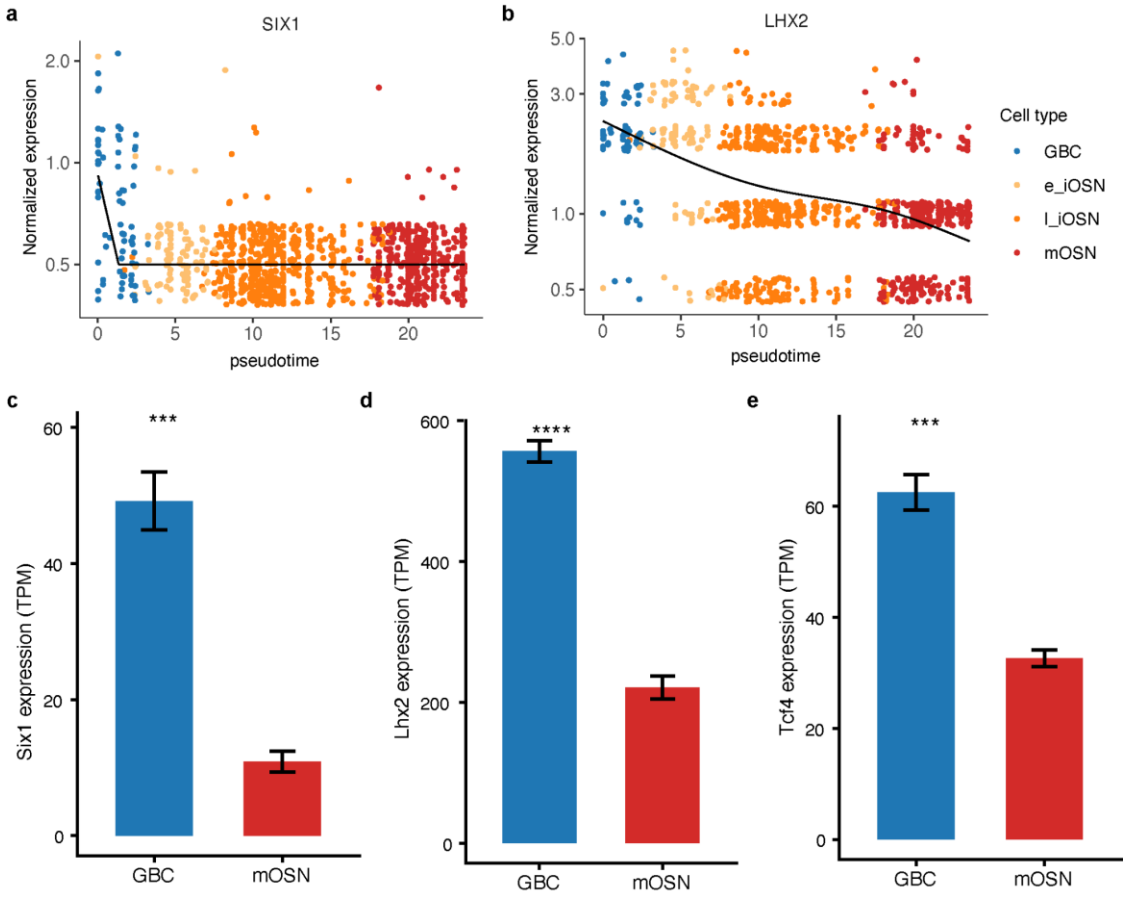
We have provided details for the re-clustering of OSNs alone: To get the cell stages of OSNs, we re-annotated the OSN cluster. Specifically, we selected 700 HVGs on cells of the OSN cluster, performed PCA reduction using the top 30 PCs, corrected batch effects using Harmony[42], and identified OSN subclusters with a resolution of 0.2.

3. In line 235 of the manuscript, it is mentioned that SIX1 and PAX6 were both highly expressed in GBCs over pseudotime. However the trend only shows that SIX1. I would not consider PAX6 highly expressed since its expression level barely goes above 0.5. Maybe it's a typo in line 237.

We thank the reviewer for the detailed comment. We have revised our manuscript and included another example, LHX2, which is highly expressed both in our snRNA cohort and in mouse GBC.

Revised main text.

> *LHX2*, a TF regulating progenitor proliferation and axon guidance[43], was highly expressed in progenitor and immature cells of both OSN and CEN lineages. The similar expression dynamic patterns of shared TFs suggest conserved roles in OSN and CEN neurogenesis (**Supplementary Fig. 17b,d**).



Supplementary Fig.17: Expression of TFs at GBCs and OSNs. a-b, The dynamic expression of *SIX1* (a) and *LHX2* **(b)** along pseudotime. **c-e**, Expression of *SIX1* (c), *LHX2* (d), and *TCF4* (e) across mouse GBCs and mOSNs that were purified using FACS. The significance was determined using the Wilcoxon test.

Reviewer #4 (Remarks to the Author):

4.1

This work is potentially very exciting, but includes fundamental problems in details at least in the present manuscript. This study uses a very small number of subjects for snRNA-seq (only 6). This reviewer is aware that, due to an urgent medical need during the COVID pandemic era, some studies with OE biopsy were published with a small number of subjects. However, the neural epithelium in the nasal cavity exists only in a limited area, and the consistency of the biopsy that meets with the goal of the present study is uncertain.

We appreciate the concern regarding the potential impact of the limited sample size on statistical power and stability. To address this comprehensively, we expanded the dataset by integrating a new independent scRNA-seq source, strictly including only normal control samples while excluding those derived from COVID-19 or presbyosmia patients to eliminate disease-related confounding effects. The total number of OSNs in the final analysis has been increased to 1,064 cells from 13 donors.

We have also performed several robustness checks, including subsampling and methodological cross-validation for trajectory inference, DEG identification, trajectory alignment between GBCs and CENs, and gene regulatory networks. Notably, none of our primary conclusions were altered after increasing the sample size or performing resampling. The core findings—specifically the OSN developmental trajectory and its capacity to track specific CEN gene dynamics—remained highly stable across all validation tests. We have added a detailed description of these stability analyses to the revised manuscript.

4.2

Furthermore, nasal cavity is highly influenced by environmental factors, whose impact may be even more robust than the impact by the genetic program. How do the authors control the influence of environmental factors on each individual? Smoking, living in urban or non-urban area (air pollution), infectious condition, potential nasal inflammation by these

factors and so on. All these are to be well controlled. to address these, in addition to molecular study, the authors may want to conduct histological study (immunohistochemistry) for each sample. More detailed demographic summary that list the potential environmental factors at each individual level will be very important. Altogether, the authors need an extensive addition regarding the data of how such environmental factors and their impact on host responses are standardized for the present study. Furthermore, data from more subjects (at least 10: ideally more) will be needed for the analysis.

We agree that smoking, air pollution, allergies, and infections can influence OE cellular states. We have provided the donor demographic, clinical, and environmental information, including age, sex, smoking status, and comorbidities (**Table 1**). All donors lived in NewYork City, reducing large geographic heterogeneity. To minimize the impact of nasal disease or prior surgery on the transcriptome, patients who had evidence or history of inflammatory sinus disease, prior surgery, or a history of anosmia were excluded.

To control for the potential confounding effects of smoking status on gene expression, we performed a variance partitioning analysis using the dreamlet package[44,45]. Genes with >5% of expression variance partitioned to smoking status (n=165) were identified and excluded from trajDEGs, ensuring that our final set of trajDEGs was robust against this covariate. These genes are mainly associated with protein modification, chromatin organization, and regulation of RNA metabolic processes (**Supplementary Fig. 6b**).

Furthermore, we also validated our key results using an independent bulk RNA-seq dataset generated from FACS-purified cells of the mouse olfactory epithelium during the critical developmental window. We validated the expression dynamics of key developmental TFs and ASD risk genes. For example, *SIX1 and LHX2*, key regulators of sensory organ development[46,47], were specifically expressed in GBCs and immature OSNs (**Supplementary Fig. 17a,c**), exhibiting the same down-trend in mouse OSNs. Besides, *SLC45A1*, an ASD risk glucose transporter gene, displayed a concordant up-trend in both the CEN and mouse and human OSN lineages. These concordances indicate that our key results are mainly driven by intrinsic biological factors rather than environmental factors.

We have revised the manuscript and discussed the limitations.

Revised main text, table, and figures.

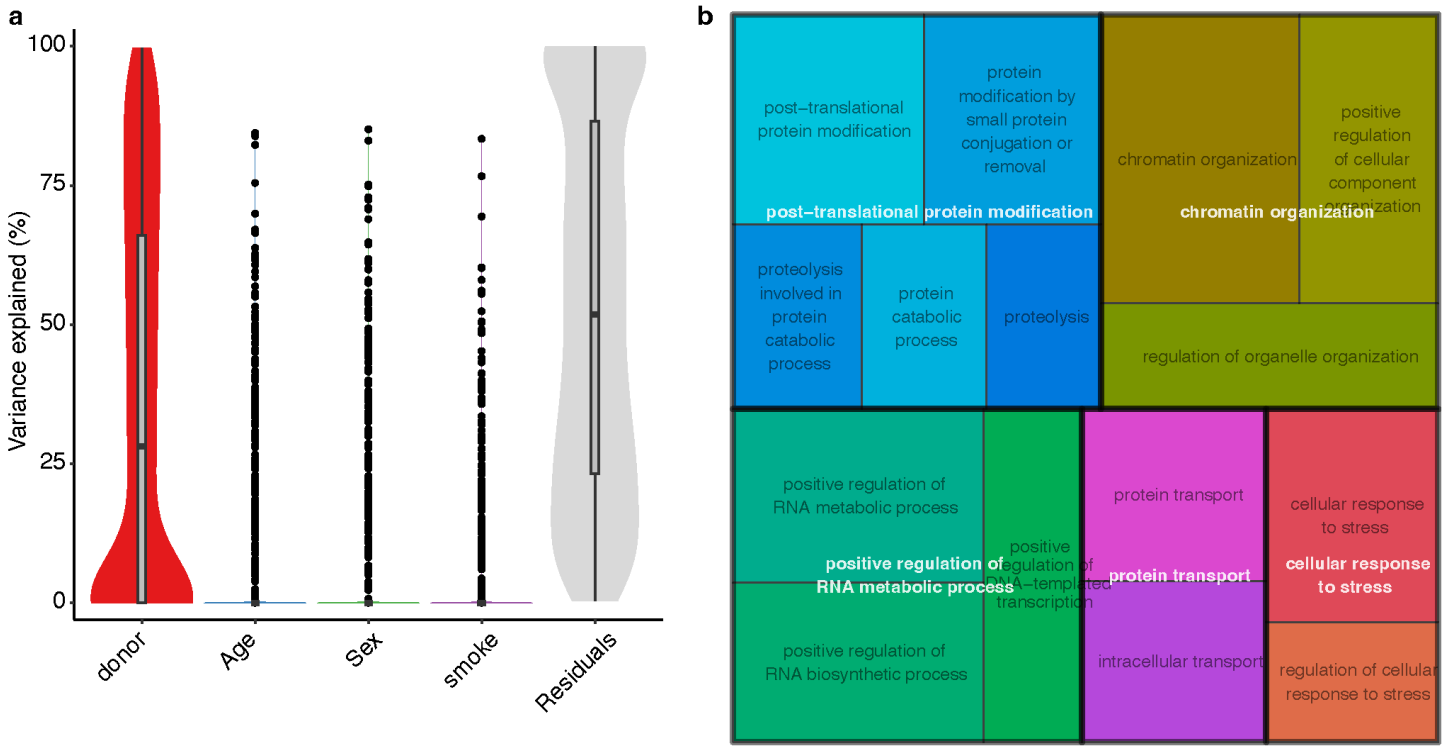
> **Results of controlling for smoking**: To further mitigate the effect of lifestyle factors (i.e., smoking status), we excluded trajDEGs with > 5% expression variance attributed to smoking, which were primarily enriched in protein modification and RNA metabolism (**Supplementary Fig. 6**).
>
> **Methods of controlling for smoking**: To control for the potential confounding effects of smoking status on gene expression, we employed a variance partitioning analysis using a linear mixed model framework implemented in the dreamlet package[44]. Genes with > 5% of expression variance partitioned to smoking status were excluded from the trajDEG set to ensure robustness against this confounding factor.
>
> **Results of validation**: Furthermore, we revealed OSN-specific TFs and TFs shared between OSN and CEN neurogenesis (**Fig. 5d-e**). For example, *SIX1*, a key regulator of sensory organ development[46,47], was specifically expressed in GBCs (**Supplementary Fig. 17a,c**). In contrast, *LHX2*, a TF regulating progenitor proliferation and axon guidance[43], was highly expressed in progenitor and immature cells of both OSN and CEN lineages; Validation against mouse fluorescence-activated cell sorting (FACS) purified GBCs and OSN datasets confirmed this concordance, with 75.5% of down-regulated ($p = 0.0051$, Hypergeometric distribution) and 53.1% ($p = 2.2e\text{-}4$, Hypergeometric distribution) of up-regulated ASD risk genes in CENs exhibiting the same trend in OSNs (**Supplementary Fig. 15**). Collectively, these results support the utility of OSNs as a model system for studying neuronal dysfunction in neurodevelopmental disorders.
>
> **Methods of validation**: To validate the dynamics of these ASD risk genes and TFs, we analyzed an independent bulk RNA-seq dataset generated from FACS-purified cells of the mouse olfactory epithelium during the critical developmental window (P2 to P16)[33]. Specifically, mature OSNs were purified from Omp-GFP mice at postnatal

stages P2, P7, and P16, while intermediate neuronal progenitors (GBC-INPs) were isolated from Neurog1-GFP mice at P3, P7, P9, and P16.

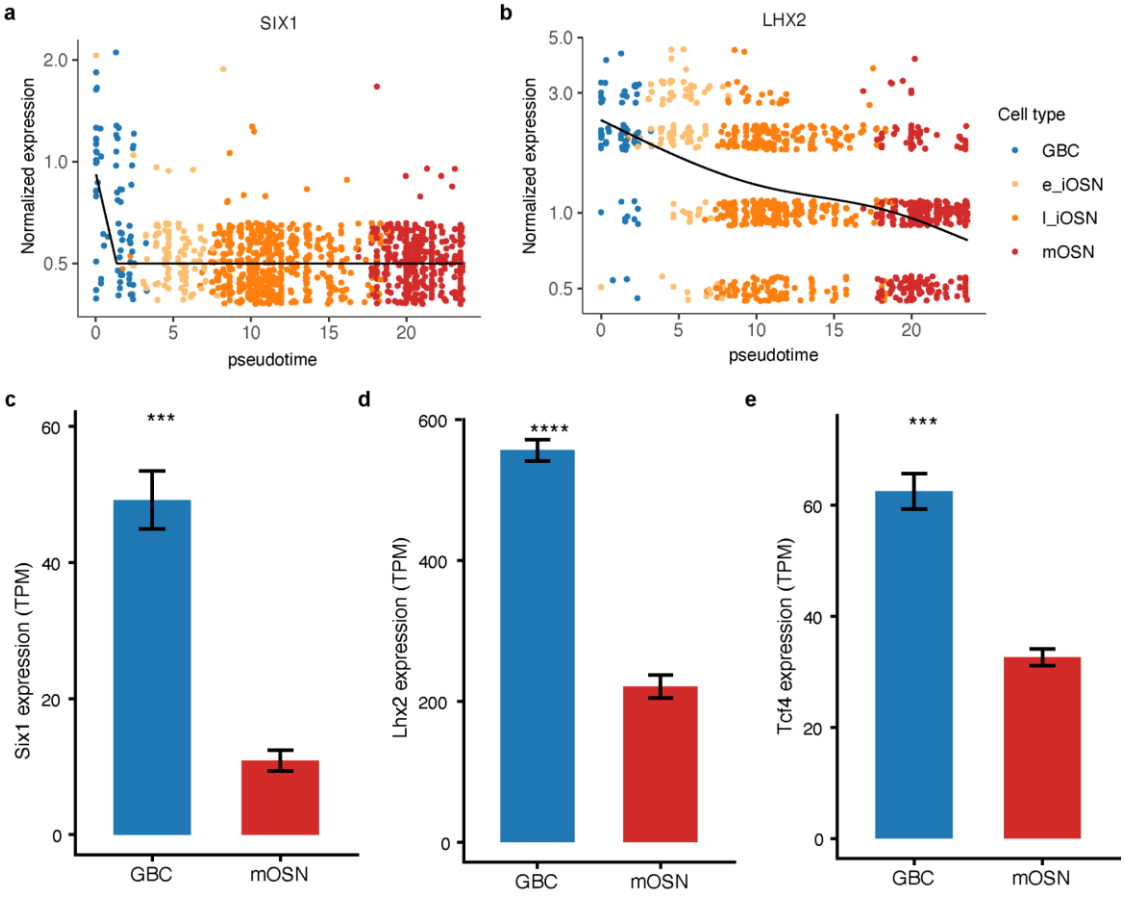
Discussion: Additionally, the nasal cavity is highly affected by environmental stressors, including air pollution and smoking[48–50]. Although we mitigated these confounding effects by carefully selecting healthy controls and removing transcripts affected by smoking status, we cannot entirely rule out the impact of individual environmental exposures. Future studies incorporating larger cohorts and systematic histological validation will be essential to standardize the host response against external environmental variables.

Table 1. Clinical information for donors.

donor_id	Age	sex	smoking status	Indication for surgery
ENT-1	42	Female	former smoker quit in 2015 (smoked for 15 years, 1/2 pack per day)	Somatotroph Pituitary macroadenoma
ENT-2	72	Male	Smokes 4 cigarettes/day x20 yrs	pituitary macroadenoma with compression of optic chiasm, as well as narrowing of cavernous ICAs
ENT-3	58	Female	never	bilateral encephalocele
ENT-5	67	Female	never	pituitary macroadenoma and CSF leak
ENT-6	34	Female	0.5 packs a day for 3 years, quit on 1/1/2010	Pituitary gland with heterogeneous enhancement with suprasellar extension, mild optic nerve compression
ENT-8	26	Male	10 years of smoking 0.25 packs a day	prolactin secreting macroadenoma



Supplementary Fig.6: Variance in gene expression attributable to key covariates. a, Distribution of the percentage of gene expression variance explained by the specified covariates (individual age, sex, and smoking status) across all analyzed genes. **b,** Functional enrichment analysis of the subset of genes with >5% of expression variance partitioned to smoking status.



Supplementary Fig.17: Expression of TFs at GBCs and OSNs. a-b, The dynamic expression of *SIX1* (a) and *LHX2* **(b)** along pseudotime. **c-e**, Expression of *SIX1* (c), *LHX2* (d), and *TCF4* (e) across mouse GBCs and mOSNs that were purified using FACS. The significance was determined using the Wilcoxon test.

4.3
The analysis itself in the present study is great.
This reviewer likes the study goal that seeks for how much neurogenesis in the olfactory neural lineage may be a good model for brain neurogenesis. However, stating that "OSNs represent a potential proxy to study gene programs involved in neurogenesis in human brains" may not be correct in the use of [OSNs] in this context. OSNs are mature neurons, and the statement should be rephrased as "cells in the olfactory neuroepithelium represent .....

We agree with the reviewer that “OSNs” is not appropriate. We have revised our manuscript.
Revised main text

Overall, cells in the olfactory neuroepithelium represent a potential proxy to study gene programs involved in neurogenesis in the human brain, providing an accessible model for investigating neurodevelopmental dysfunction in psychiatric disorders.

4.4
In all, I admit the potential of this project. The final goal that the authors aim is great. Nevertheless, at least at present, the fundamental problems exist. The central issue of how much each individual (even called as healthy subjects) is very different with each other under daily influence of environmental factors is missing in this manuscript. In the nasal cavity, environmental factors that happen to each individual in different manners may influence gene expression, which may be even more than the impact by the intrinsic genetic factors. Accordingly, the authors may be expected to conduct an extensive, long-term revision if they wish.

We agree that the olfactory epithelium, being directly exposed to the external environment, is susceptible to environmental influences that can induce transcriptomic variation. As stated above, we have addressed this through a combination of stringent clinical recruitment, robust statistical filtering, cohort extension, and robustness validation. These ensure that the transcriptomic signatures reported in our study represent robust biological programs of OSN development rather than transient environmental responses.

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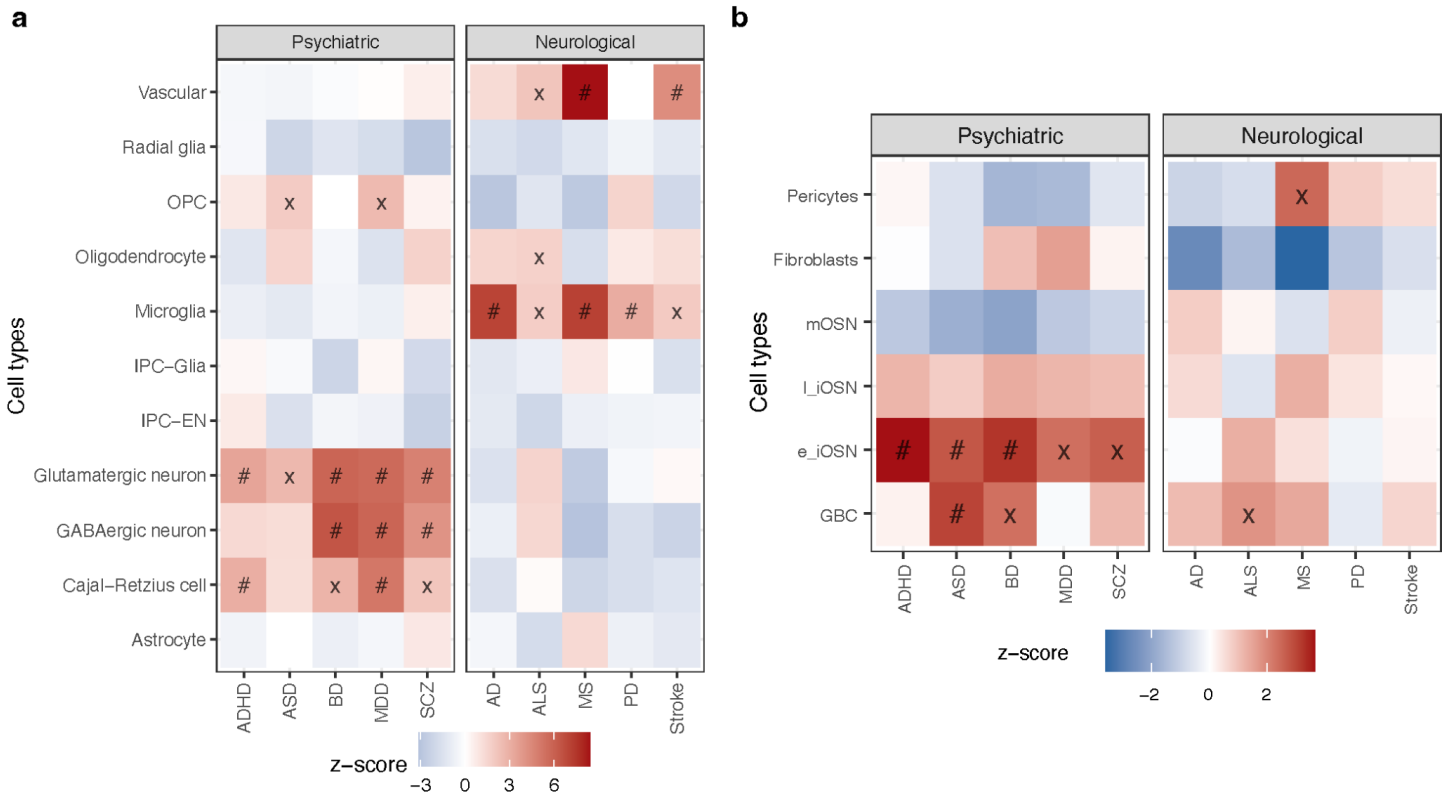
Reviewer #1 (Remarks to the Author):

The authors have taken appropriate steps to address my previous comments. The revisions have improved the clarity of methods and results. Here are additional suggestions:

1. Cell-type relevance to genetic risk assessed using scDRS
-- It would be helpful to include prefrontal cortex neurons as a positive control, and fibroblasts and pericytes from the olfactory epithelium as negative controls, to better contextualize the scDRS findings.

We thank the reviewer for the suggestion. We performed additional scDRS association analyses to include cortical neurons as positive controls and non-neuronal cell types from the olfactory epithelium (OE), such as fibroblasts and pericytes, as negative controls (**Supplementary Fig. 5**). As expected, glutamatergic and GABAergic cortical neurons showed significant associations with psychiatric disorders. In contrast, fibroblasts and pericytes did not show significant associations. These results provide additional context for interpreting the scDRS signals observed in OSNs.



Supplementary Fig. 5: The single-cell disease relevance score (scDRS) of brain-related diseases across cells in the cortex and olfactory epithelium (OE). a, scDRS association between cortex cells and neuropsychiatric disorders. **b**, scDRS association between OE cells and neuropsychiatric disorders. The color represents the Monte Carlo (MC) z-score for their association. ADHD: Attention-deficit/hyperactivity disorder, ASD: Autism spectrum disorder, BD: Bipolar Disorder, MDD: Major Depressive Disorder, SCZ: Schizophrenia, AD: Alzheimer's Disease, ALS: Amyotrophic lateral sclerosis, MS: Multiple Sclerosis, PD: Parkinson's Disease. # and x represent fdr < 0.05 and p < 0.05, respectively.

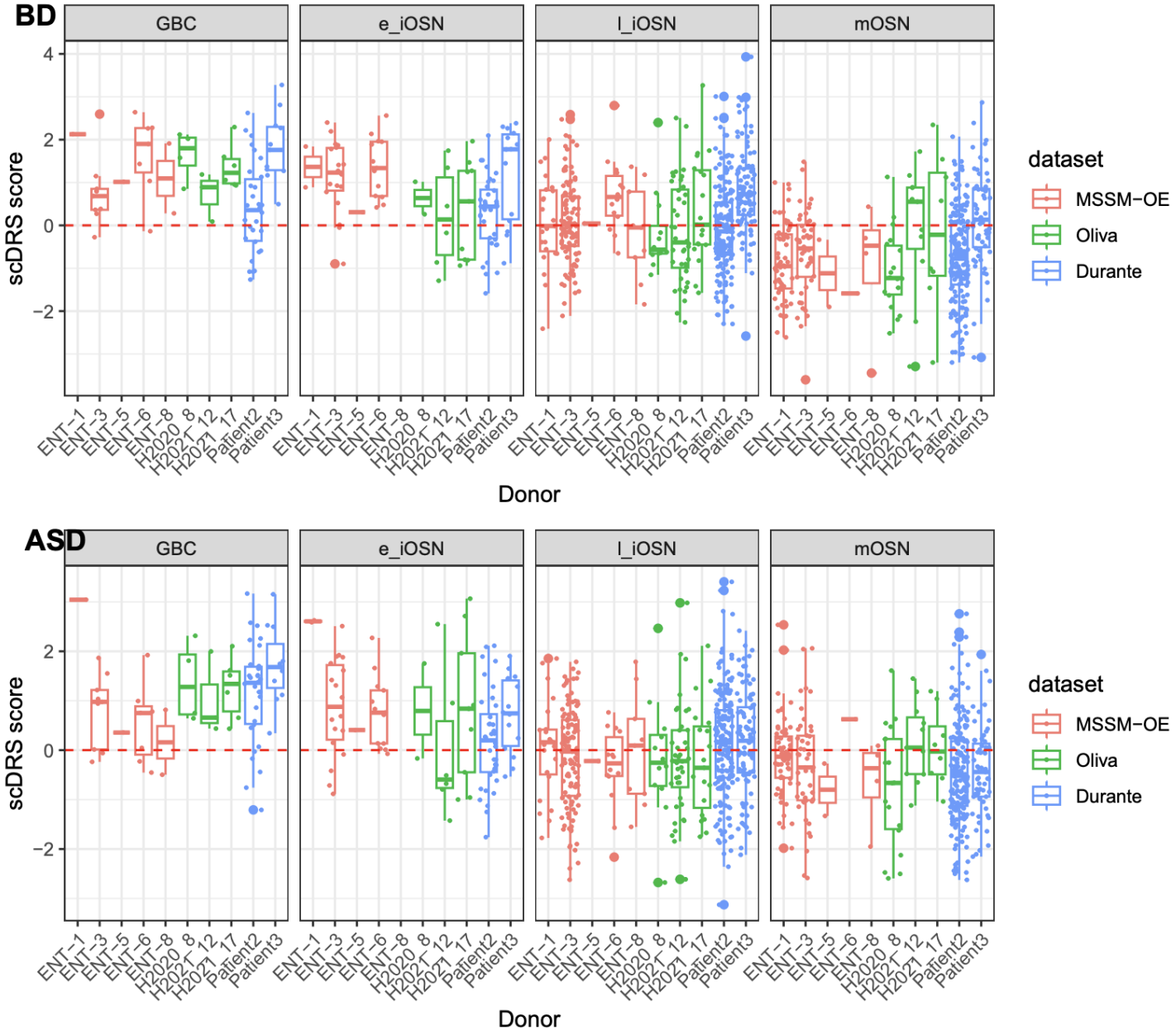
Revised manuscript:

As negative controls, fibroblasts and pericytes did not show any associations with neuropsychiatric disorders.

-- I am also curious about the contribution of mOSN, e_iOSN, and l_iOSN cells from each participant. If these cell types are unevenly represented across subjects, inter-individual differences rather than cell-type–specific effects could underlie the observed genetic risk associations.
-- Another issue is that GBC and e_iOSN—the two cell types with the smallest sample sizes—showed significant enrichment. If these cells were derived from fewer subjects, whereas mOSN and l_iOSN were contributed by a larger and

more diverse set of participants, greater inter-individual heterogeneity in mOSN and l_iOSN could potentially dilute the signal and contribute to the lack of significance.

Following your suggestion, we checked the distribution of single-cell disease relevance score (scDRS) across datasets and individual donors (**Supplementary Fig. 6**). As representative examples, we present ASD and BD, which show significant associations with GBC and e_iOSNs. Although some inter-individual heterogeneity was observed, the overall pattern was highly consistent across donors. Specifically, in most individuals, GBCs and iOSNs exhibited higher scDRS scores, whereas mOSNs consistently showed lower scores. Higher scores (values > 0) indicate stronger expression of disease-associated genes. The residual inter-individual variability may partially reflect technical differences across datasets, snRNA-seq of the MSSM-OE versus scRNA-seq of the Durante and Oliva datasets. In summary, despite these potential sources of heterogeneity, the relative pattern of higher scores in GBC/iOSNs and lower scores in mOSNs was reproducible across donors.



Supplementary Fig. 6: Distribution of scDRS score across datasets and donors. a, scDRS score between GBC/OSN and neuropsychiatric disorders. Each point represents a single cell.

2. Similarities and differences between OSNs and CENs.

-- The third and fourth paragraphs in this subsection are complex, and the results are difficult to interpret. Several definitions and cutoffs are unclear, and some conclusions may be overinterpreted. I suggest that the authors either clarify key details and temper some interpretations, or shorten these sections and move part of them to the supplementary materials.

We thank the reviewer for the suggestion. We have revised this section to improve clarity and provide additional methodological details in the Methods section. We found that the gene-level enrichment analyses largely recapitulated the pathway-level results, introducing redundancy and complexity of the original presentation. To simplify the interpretation and reduce the redundancy, we removed the gene-level enrichment analyses and retained the pathway-level results in the revised manuscript.

Revised manuscript:

> **Main text**: To further investigate the developmental similarities between OSNs and CENs, we compared their trajDEGs and dynamic biological processes. trajDEG overlap was defined as genes that were dynamically expressed in both OSNs and CENs within the same trend category. Down-trending genes showed the greatest overlap (**Fig. 3c**, Fisher's exact test), suggesting relatively greater similarity during earlier developmental stages, corresponding to the GBC-to-iOSN transition in OSNs and the fetal-to-infant stage in CENs. We next compared the significance of the top 10 enriched biological processes (**See Methods**), revealing three distinct patterns across different DEG trends (**Fig. 3d-f and Supplementary Fig. 14**). For down-trending genes (**Fig. 3d**), most processes, including neurogenesis, RNA metabolism regulation, and cell proliferation, were highly enriched in both OSNs and CENs. For trans_up genes (**Fig. 3e**), axon development and synapse organization were enriched in both cell types. For up-trending genes (**Fig. 3f**), the processes exhibited cell type specificity associated with functional specialization. Specifically, OSNs were enriched in cilium organization and cilium movement, while CENs were associated with synaptic signaling. These findings suggest partial convergence of early developmental programs between OSNs and CENs, followed by increasing divergence during later stages of maturation.
>
> **Methods**: TrajDEGs of CENs were identified using the same pipeline. To examine the overlap of trajDEGs between OSN and CEN, Fisher's exact test was applied.
> Enriched biological processes were ranked based on adjusted p-values (FDR). To facilitate comparison between the two cell types, we selected the top 10 significantly enriched biological processes (FDR < 0.05) for OSNs and CENs within each trend category.

-- Line 214: How was the "overlap between OSNs and CENs" defined?

The overlap of trajDEGs was defined as genes that were identified as dynamically expressed in both OSN and CEN trajectories and assigned to the same dynamic trend category. We have added this information to our results.

-- Line 215: The statement "stronger convergence during early developmental stages" may be overstated. CENs were from which developmental stage?

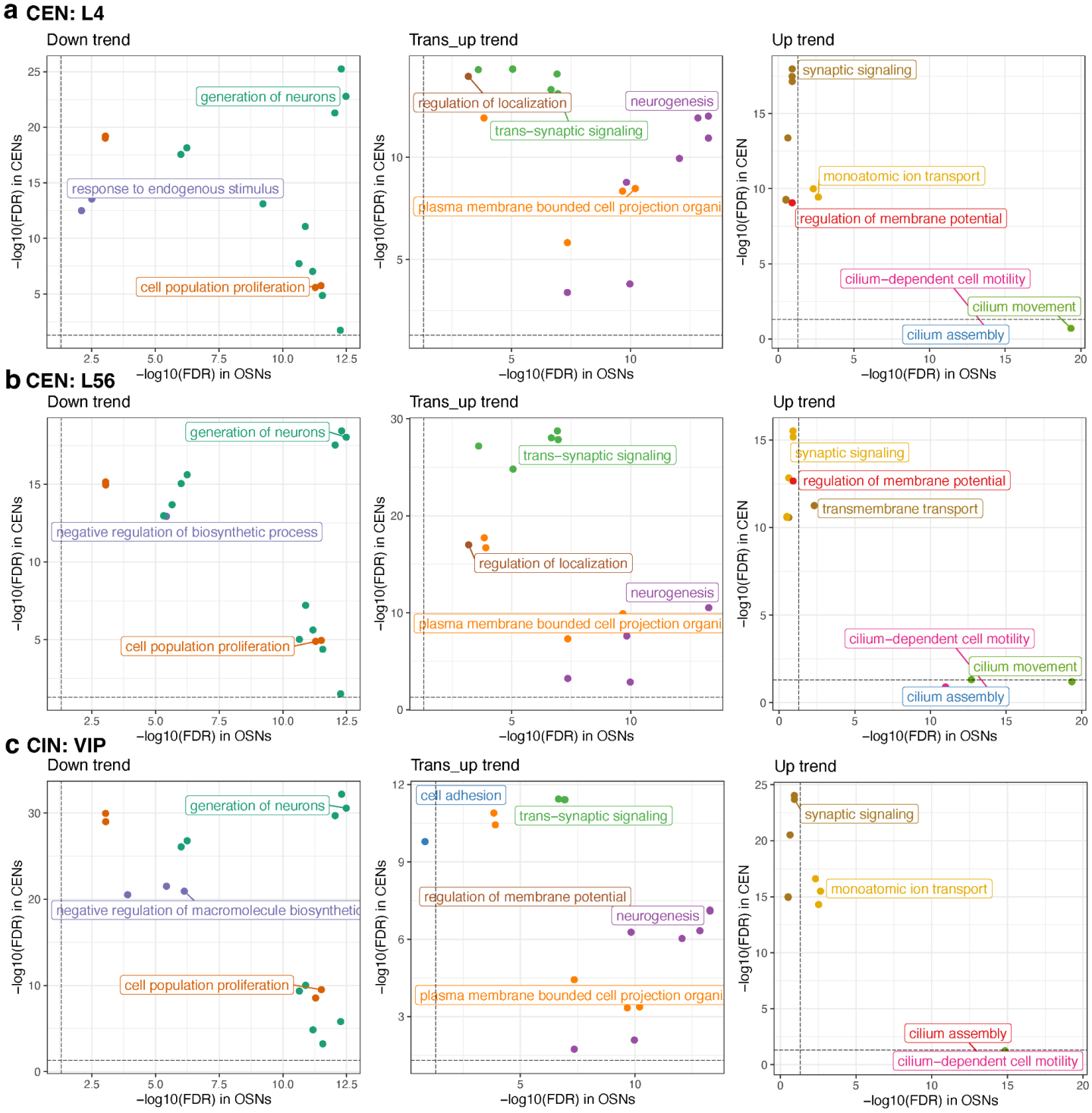
We thank the reviewer for the comment. We have revised our text as follows:

Revised main text:

> Down-trending genes showed the greatest overlap (**Fig. 3c**, Fisher's exact test), suggesting relatively greater similarity during earlier developmental stages, corresponding to the GBC-to-iOSN transition in OSNs and the fetal-to-infant stage in CENs.

-- Line 216: What is the justification for selecting layer 2/3 neurons as the reference?

We selected layer 2/3 excitatory neurons as the reference for several reasons. First, transcriptional programs are broadly similar across cortical excitatory neuron subtypes, and our preliminary analyses showed that the overlap patterns of trajDEGs were highly consistent across layers (**Fig. 3c**). Second, layer 2/3 neurons represent the largest population in the dataset, providing greater statistical robustness for downstream comparisons. Third, their transcriptional profiles showed the highest similarity to OSNs among excitatory neuron subtypes (**Fig. 3a**). To ensure that our conclusions were not dependent on this choice, we additionally performed the same analyses using other cortical layers (**Supplementary Fig. 14**), which yielded highly consistent results. We have revised our main text to increase its clarity and robustness.



Supplementary Fig. 14: Significance of enriched biological processes for trajDEGs in OSNs compared with trajDEGs in cortical neurons. Panels a–c correspond to L4 CENs, L5/6 CENs, and VIP CINs, respectively.

-- Lines 218–222: How were gene sets such as shared-down, shared trans-up, and shared up-regulated defined?

For both OSNs and CENs, we first identified genes showing significant dynamic changes along the pseudotime trajectory (trajDEGs). Based on their expression dynamics, these genes were classified into three patterns: down-trending, trans_up, and up-trending. “Shared” gene sets were defined as genes exhibiting the same dynamic trend in both OSN and CEN trajectories. For example, shared-down genes refer to genes that showed decreasing expression during both OSN and CEN development. Similarly, shared trans_up genes showed transient upregulation in both trajectories, and shared up-regulated genes showed increasing expression during differentiation in both cell types.

-- Line 225: How were the “top-enriched biological processes” defined? What cutoff was used to determine “top”?

For each dynamic trend category, we performed Gene Ontology enrichment analysis separately for OSN and CEN gene sets. Enriched biological processes were ranked based on adjusted p-values (FDR). To facilitate comparison between the two cell types, we selected the top 10 significantly enriched biological processes (FDR < 0.05) for OSNs and CENs within each trend category. We have revised our methods section to increase clarity.

Revised methods:

> Functional enrichment of GO biological processes was performed using gprofiler2 (v0.2.3)[1]. For trajDEG of OSNs and CENs, custom backgrounds of genes expressed in more than five OSNs and CENs, respectively, were applied. Multiple testing corrections were performed using FDR, with significant enrichments identified at FDR < 0.05. Terms with < 2000 genes were selected to focus on more informative and interpretable terms.
>
> Enriched biological processes were ranked based on adjusted p-values (FDR). To facilitate comparison between the two cell types, we selected the top 10 significantly enriched biological processes (FDR < 0.05) for OSNs and CENs within each trend category. The rrvgo (v1.14.2) package was applied to reduce the redundancy of GO lists by grouping similar terms based on their semantic similarity[2]. Terms with the highest enrichment significance were picked to represent each group.

-- Line 226: Is this comparing enrichment results of down-trending genes in OSNs with enrichment results of all genes in CENs?

We thank the reviewer for the opportunity to clarify. We didn’t compare that with all genes. Instead, we identified dynamically expressed genes (trajDEGs) separately for both OSNs and CENs and classified them into different dynamic trends. The enrichment analyses were performed within the same trend category for each cell type. Specifically, for this comparison, we analyzed enrichment of down-trending genes in OSNs and down-trending genes in CENs, rather than comparing down-trending genes in OSNs with all genes in CENs.

-- Lines 233–234: These statements appear to be overclaimed.

We have revised as follows:

Revised text:

> These findings suggest partial convergence of early developmental programs between OSNs and CENs, followed by increasing divergence during later stages of maturation.

Minor comments:

-- It would be helpful if the authors could report the sample sizes of GBC and e_iOSN.

We have added the number of cells for each cell type.

Revised text:

> Within the combined dataset (n = 1,064), consisting of 74 GBCs, 601 iOSNs, and 389 mOSNs, cells were predominantly clustered by developmental stage rather than data source.

-- There are discrepancies between the tracked-changes manuscript and the clean version. For example, the tracked-changes version (line 535) has “The marker gene lists were obtained downloaded from Wang et al.’s data 34” whereas the clean version states “The marker gene lists were obtained from Wang et al.’s data 37”.

Thanks for pointing this out. We have corrected this discrepancy and ensured that the tracked changes and clean versions are now consistent.

-- Fig. 4e, while the inclusion of an independent mouse dataset is valuable, why not also compare gene expression across GBC, e_iOSN, l_iOSN, and mOSN using the dataset analyzed in this study?

The independent mouse dataset included in Fig. 4e contains only two annotated cell populations, GBCs and mOSNs, and does not include intermediate neuronal populations corresponding to e_iOSNs or l_iOSNs. Therefore, we did not compare gene expression across GBC, e_iOSN, l_iOSN, and mOSN.

Reviewer #2 (Remarks to the Author):

Authors almost solved all my concerns and here I recommended the acceptance of this manuscript for publication. One more suggestion is regarding my previous comment 2.7. Authors performed bulk RNA-Seq analysis on mature OSNs and GBCs at P2-P16 to validate the expression of ASD risk genes. While these experiment data were solid, it is better to show the expression changes at protein level.

We thank the reviewer for this suggestion. While protein-level validation would further strengthen the findings, such experiments require additional human olfactory epithelium samples across developmental stages and are therefore beyond the scope of the current study. We have added this point as a limitation in the Discussion.

Revised discussion:

> In addition, although we validated the expression dynamics of some critical TFs and ASD risk genes using mouse RNA-seq data, further systematic experimental validation, at both transcription and protein levels in human tissue contexts, would strengthen the robustness of our findings.

Reviewer #3 (Remarks to the Author):

Although some limitations remain due to sample size, the authors did a good job responding to prior critiques. Supported by welcomed clarification, numerous additional analyses, and thoughtful responses to reviewer points, I think the study/manuscript is certainly of quality for publication in Nature Communications. I endorse acceptance.

Reviewer #4 (Remarks to the Author):

The authors are very responsive to the questions and requests from this reviewer. Thus, this reviewer finds the present revision as successful in principle. Based on this progress, this reviewer will underscore the following two points and expect the authors to be continuously responsive.

First point:
In the original manuscript, the authors tended to highlight only OSNs in their statement. However, this reviewer provided a comment on this concern, to which the authors made a good revision (see below)

4.3, However, stating that "OSNs represent a potential proxy to study gene programs involved in neurogenesis in human brains" may not be correct in the use of [OSNs] in this context. OSNs are mature neurons, and the statement should be rephrased as "cells in the olfactory neuroepithelium represent .....

Nevertheless, there remains inaccurate sentences because of limited highlight to OSNs in the revised manuscript. For example: “increasing evidence showed that the olfactory epithelium is directly affected (functional and molecular alterations in OSNs) in neuropsychiatric disorders16,17,24.” Including these studies, several groups have successfully used olfactory epithelium-derived neuronal cells in demonstrating the pathophysiology of the olfactory epithelium in neuropsychiatric conditions, but these may not be attributed only to OSNs. In many lab studies, indeed, olfactory epithelium-derived neuronal cells have been frequently used, but these may not be exactly OSNs. In short, the problem of the example above is resolved by removing the phrase of (……). The authors may want to revisit the entire manuscript to avoid similar mistakes.

Thanks for the suggestion. We have revised the manuscript to replace “OSNs” with “olfactory epithelium–derived neuronal cells” when referring to previous studies, to more accurately describe the cell populations used in those reports.

We agree that mature OSNs themselves do not represent the entire neurogenic process in the olfactory epithelium, which involves multiple stages of the neuronal lineage. In the original text, we intended to refer to neuronal cells within the olfactory epithelium, including progenitors and immature neurons, rather than mature OSNs alone. To avoid potential confusion, we have revised the phrase in the manuscript.

Revised manuscript:

> We identified distinct developmental stages of the OSN lineage, including GBCs, as well as immature and mature OSNs.
>
> Through systematic characterization of gene expression and regulatory dynamics across the developmental trajectory of OSN lineage, we elucidated critical biological processes underlying neurogenesis and neuronal maturation, achieving a finer resolution than with bulk data[3].
>
> Cells in the neuronal lineage of the OE represent a potential proxy for studying gene programs involved in neurogenesis in the human brain.
>
> Our study provides a novel perspective on the transcriptional dynamics of cells in the neuronal lineage of the OE, demonstrating their potential as a model system for investigating neurodevelopmental processes and disease-associated gene expression.

Second point:
As far as this reviewer sees, at least one reviewer finds some concern in references. Indeed, several representative papers in this field seem to be missing. In particular, given that the merit of studying neuronal signatures from the olfactory epithelium rather than iPS cells (reprogramed cells) is the potential of disease-associated, development/aging-associated epigenetic signatures (F Gage and several others have highlighted the significance of non-reprogrammed neuronal cells in research), the past works related to epigenetic landscapes and development/aging-associated process are important. Accordingly, this reviewer recommends the authors to include:
- Mol Psychiatry. 2013 Jul;18(7):740-2. PMID: 22925834.
- Schizophrenia (Heidelb). 2025 Apr 23;11(1):68. PMID: 40268926.

Regarding the increasing evidence showing the olfactory epithelium is directly affected in neuropsychiatric disorders, the current three citations are appropriate. This reviewer recommends the authors to cite the following papers in this citation.
- Mol Psychiatry. 2024 May;29(5):1453-1464. PMID: 38321120, PMCID: PMC11189720
- Transl Psychiatry. 2018 Apr 18;8(1):81. PMID: 29666369.

We thank the reviewer for the suggestion. We have added these references.

Revised manuscript:

Although induced pluripotent stem cell (iPSC)-based disease models could be an alternative approach that might overcome these limitations, they erase disease-associated, development/aging-associated processes and epigenetic signatures[4,5].

Accordingly, OSNs have been utilized to unveil the neuronal signatures of psychiatric and neurological disorders such as schizophrenia, depression, bipolar disorder, Alzheimer's disease (AD), and Parkinson's disease[6–10]. For instance, dysregulation of Wnt signaling, microRNA-124-3p, and histone methylations have been observed in schizophrenia patient-derived OSNs[11–16].

References

1. Kolberg, L., Raudvere, U., Kuzmin, I., Vilo, J. & Peterson, H. gprofiler2 -- an R package for gene list functional enrichment analysis and namespace conversion toolset g:Profiler. *F1000Res.* **9**, 709 (2020).
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4. Mertens, J. *et al.* Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell* **17**, 705–718 (2015).
5. Frobel, J. *et al.* Epigenetic rejuvenation of mesenchymal stromal cells derived from induced pluripotent stem cells. *Stem Cell Reports* **3**, 414–422 (2014).
6. Marin, C., Alobid, I., Fuentes, M., López-Chacón, M. & Mullol, J. Olfactory dysfunction in mental illness. *Curr. Allergy Asthma Rep.* **23**, 153–164 (2023).
7. Yang, K. & Evgrafov, O. V. Editorial: Olfactory neuroepithelium-derived cellular models to study neurological and psychiatric disorders. *Front. Neurosci.* **17**, 1203466 (2023).
8. Yang, K., Ishizuka, K., Tomoda, T. & Sawa, A. Aberrant aging-associated p62 autophagic cascade in biopsied olfactory neuronal cells from patients with psychosis. *Schizophrenia (Heidelb.)* **11**, 68 (2025).
9. Yang, K. *et al.* Inflammation-related pathology in the olfactory epithelium: its impact on the olfactory system in psychotic disorders. *Mol. Psychiatry* **29**, 1453–1464 (2024).
10. McLean, C. K. *et al.* Lithium-associated transcriptional regulation of CRMP1 in patient-derived olfactory neurons and symptom changes in bipolar disorder. *Transl. Psychiatry* **8**, 81 (2018).
11. Lavoie, J., Sawa, A. & Ishizuka, K. Application of olfactory tissue and its neural progenitors to schizophrenia and psychiatric research. *Curr. Opin. Psychiatry* **30**, 176–183 (2017).
12. Son, G. *et al.* Olfactory neuropathology in Alzheimer's disease: a sign of ongoing neurodegeneration. *BMB Rep.* **54**,

295–304 (2021).

13. Evgrafov, O. V. *et al.* Gene expression in patient-derived neural progenitors implicates WNT5A signaling in the etiology of schizophrenia. *Biol. Psychiatry* **88**, 236–247 (2020).
14. English, J. A. *et al.* Reduced protein synthesis in schizophrenia patient-derived olfactory cells. *Transl. Psychiatry* **5**, e663 (2015).
15. Namkung, H. *et al.* The miR-124-AMPAR pathway connects polygenic risks with behavioral changes shared between schizophrenia and bipolar disorder. *Neuron* **111**, 220–235.e9 (2023).
16. Kano, S. *et al.* Genome-wide profiling of multiple histone methylations in olfactory cells: further implications for cellular susceptibility to oxidative stress in schizophrenia. *Mol. Psychiatry* **18**, 740–742 (2013).

Reviewer #4 (Remarks to the Author):

The authors are responsive to reviewers' comments. I appreciate the potential of this manuscript.

Two remaining issues.

1) Although the authors stated the revision of the text with the additional references in their response letter, these changes are not reflected in the main text. This reviewer regards this as a mere mistake by the authors. However, such sloppy work is not appreciated. Please fix this problem.

We apologize for the previous typo error. We have added the additional references in the main text.
Revised manuscript:

> Although induced pluripotent stem cell (iPSC)-based disease models could be an alternative approach that might overcome these limitations, they erase disease-associated, development/aging-associated processes and epigenetic signatures[1,2].
>
> Accordingly, OSNs have been utilized to unveil the neuronal signatures of psychiatric and neurological disorders such as schizophrenia, depression, bipolar disorder, Alzheimer's disease (AD), and Parkinson's disease[3–7]. For instance, dysregulation of Wnt signaling, microRNA-124-3p, and histone methylations have been observed in schizophrenia patient-derived OSNs[8–13].

2) In addition, a recent paper published in Nature Communications is to be mentioned to enhance the discussion regarding the utility of human olfactory epithelium in brain disorder research.
Olfactory cleft biopsy analysis of Alzheimer's disease pathobiology across disease stages.
D'Anniballe VM, Kim S, Finlay JB, Wang M, Ko T, Luo S, Whitson HE, Johnson KG, Goldstein BJ.
Nat Commun. 2026 Mar 18;17(1):2245. doi: 10.1038/s41467-026-70099-7.
PMID: 41851126

We thank the reviewer for the suggestion. We have added this reference.
Revised manuscript:

> Similarly, neuroinflammatory changes in olfactory neurons and immune cells were detected even in pre-clinical AD subjects[14].

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3. Marin, C., Alobid, I., Fuentes, M., López-Chacón, M. & Mullol, J. Olfactory dysfunction in mental illness. *Curr. Allergy Asthma Rep.* **23**, 153–164 (2023).
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13. Kano, S. *et al.* Genome-wide profiling of multiple histone methylations in olfactory cells: further implications for cellular susceptibility to oxidative stress in schizophrenia. *Mol. Psychiatry* **18**, 740–742 (2013).

14. D'Anniballe, V. M. *et al.* Olfactory cleft biopsy analysis of Alzheimer's disease pathobiology across disease stages. *Nat. Commun.* **17**, 2245 (2026).