

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

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Center for Disease Neurogenomics

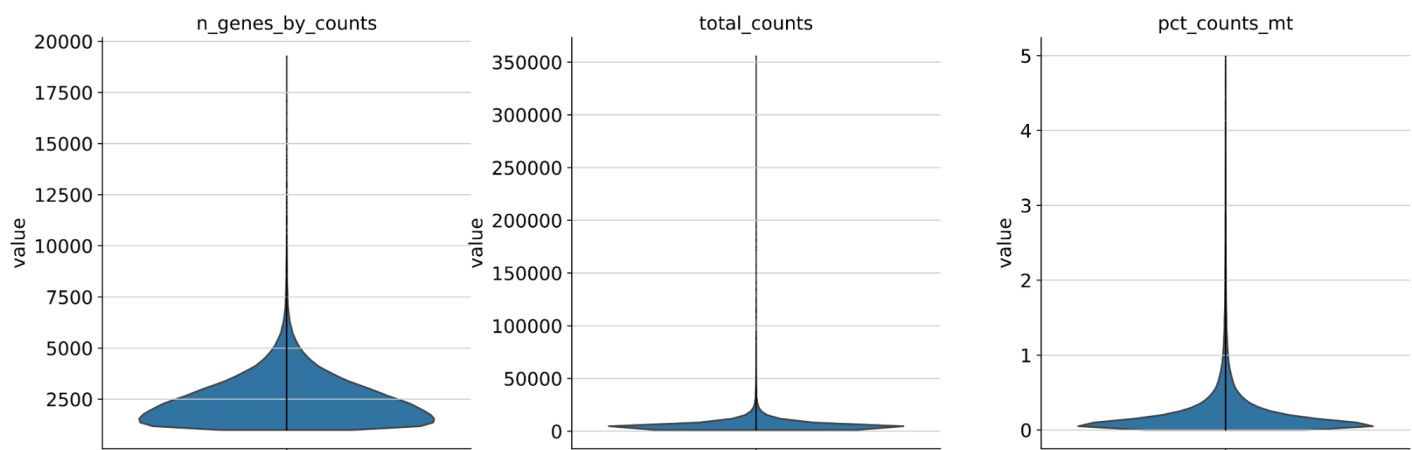
Department of Psychiatry and Friedman Brain Institute

Department of Genetics and Genomic Sciences and Institute for Data Science and Genomic Technology

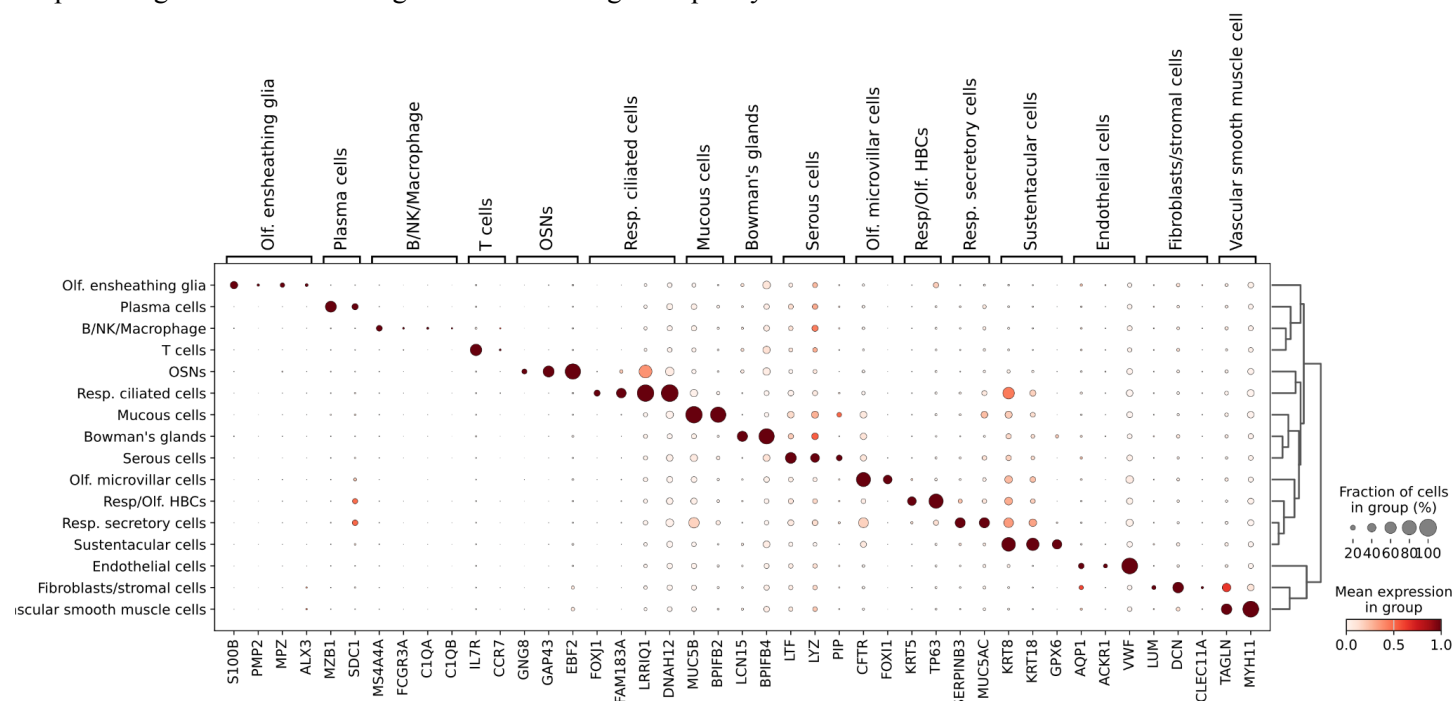
Icahn School of Medicine at Mount Sinai

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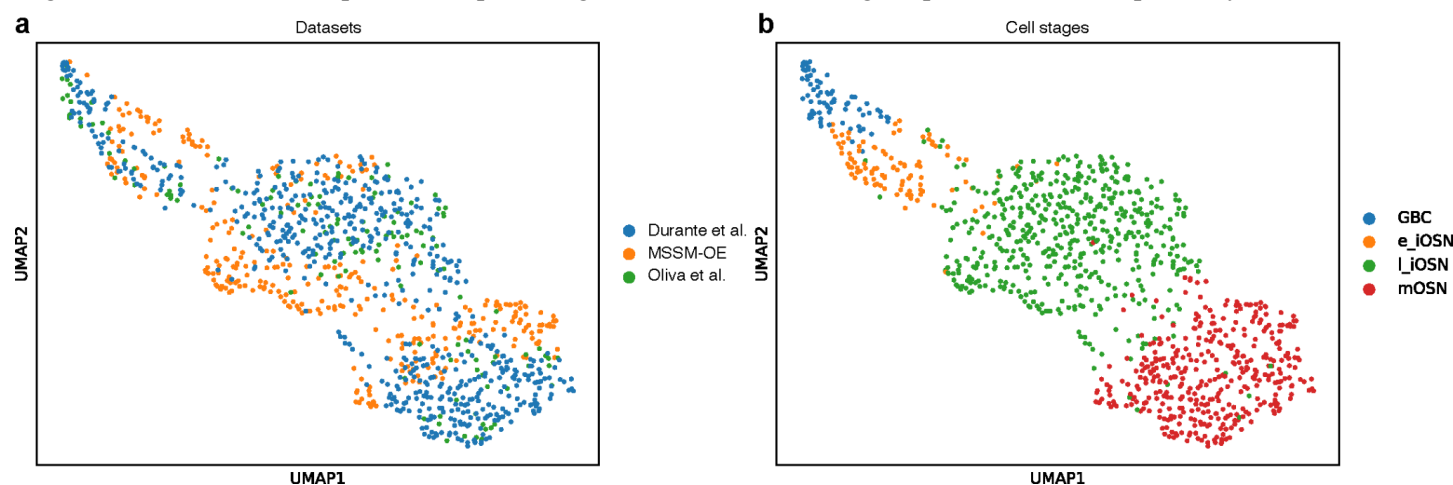
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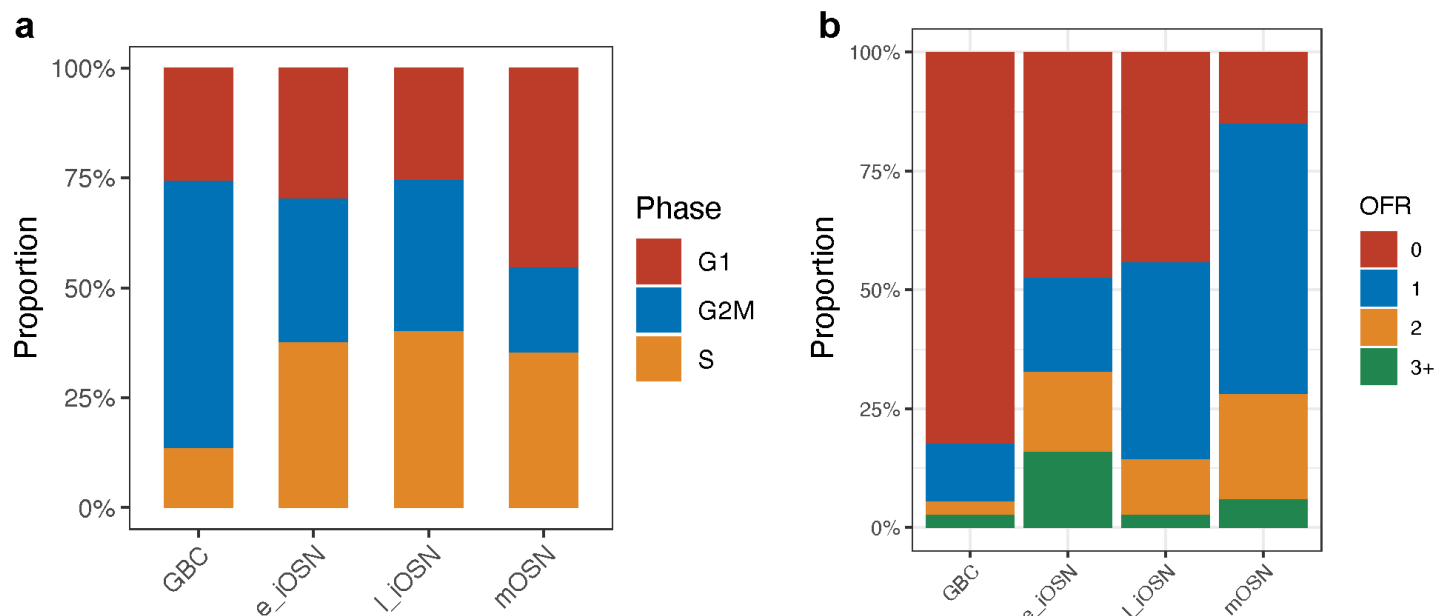
Supplementary Fig. 1: Quality control of snRNA-seq data. The distribution of the number of genes, total counts, and the percentage of mitochondrial genes after filtering low-quality cells.



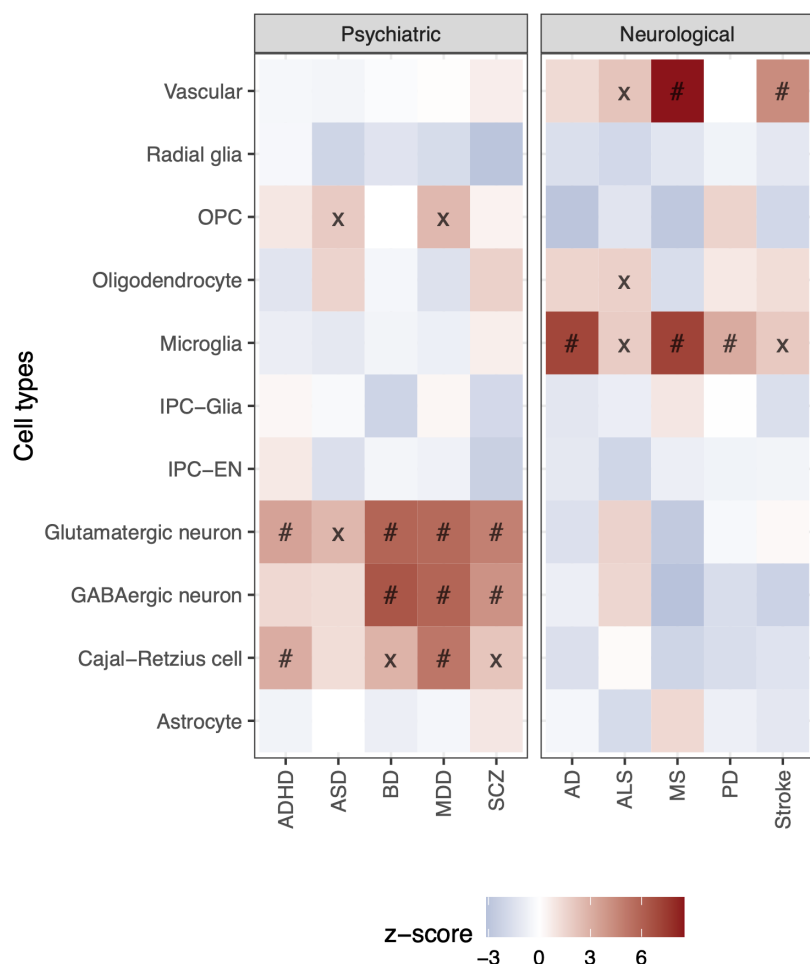
Supplementary Fig. 2: Expression of marker genes across cell clusters. Expression levels of key markers across OSN stages. Dot size and color represent the percentage of cells and the average expression level, respectively.



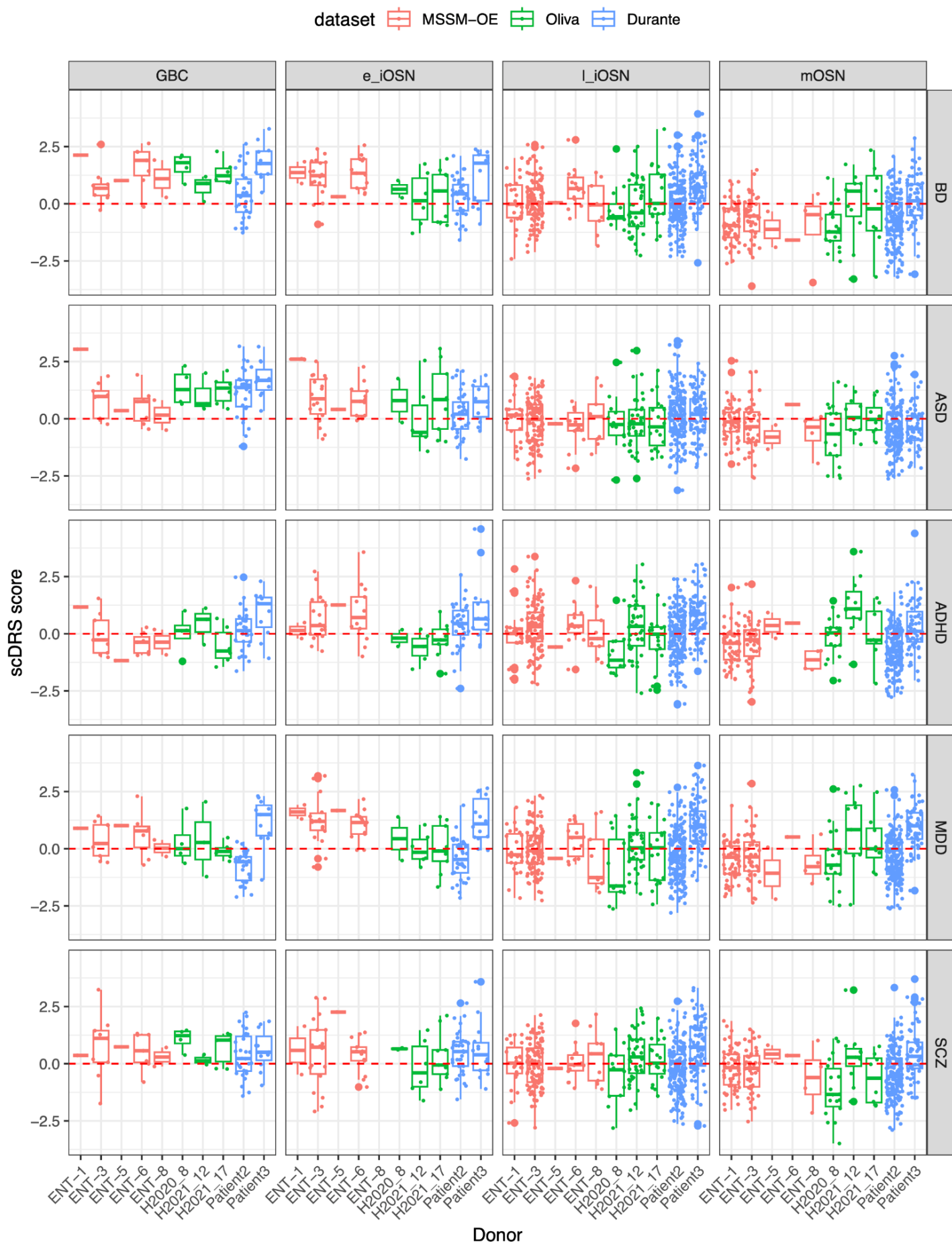
Supplementary Fig. 3: UMAP visualizations of integrated GBCs and OSNs across cohorts. a-b, GBCs and OSNs from the current study (MSSM-OE), Durante *et al.*'s cohort¹, and Oliva *et al.*'s cohorts² were integrated using scVI and visualized via UMAP. Nuclei are colored by (a) data source and (b) developmental cell stage.



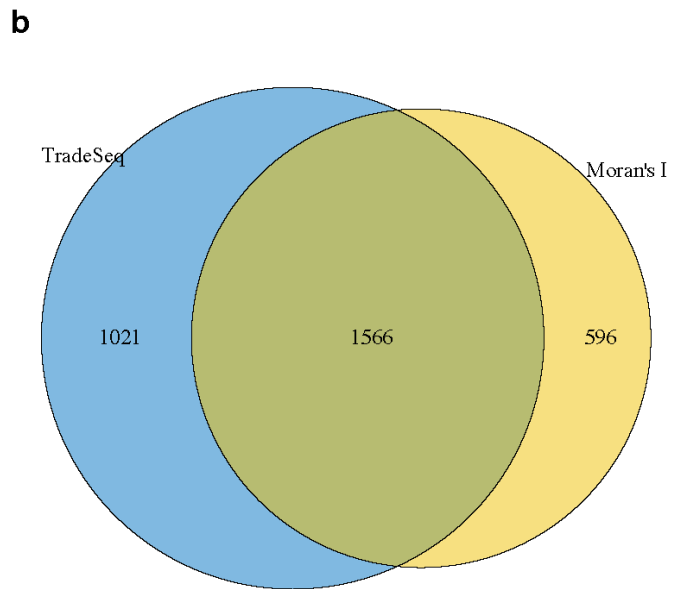
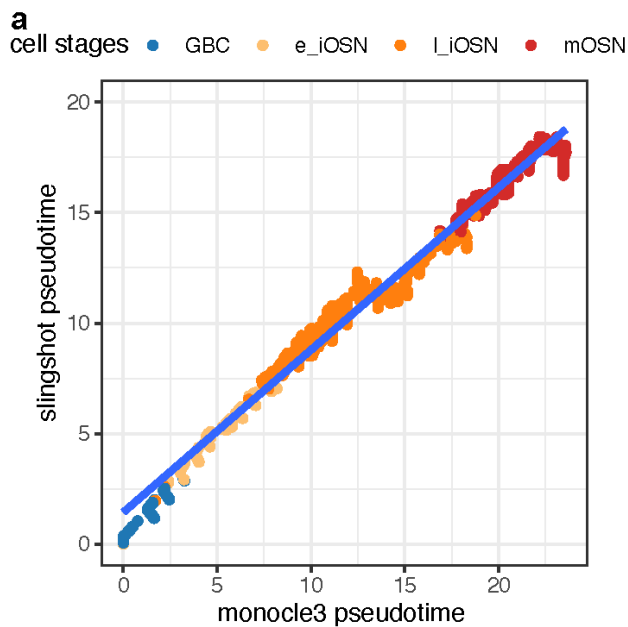
Supplementary Fig. 4: Distribution of cell cycle phases and olfactory receptor (OFR) expression across the OSN lineage. a, Percentage of cells in each cell cycle phase across cell stages. **b,** Proportion of cells expressing varying numbers of olfactory receptor genes across cell stages.



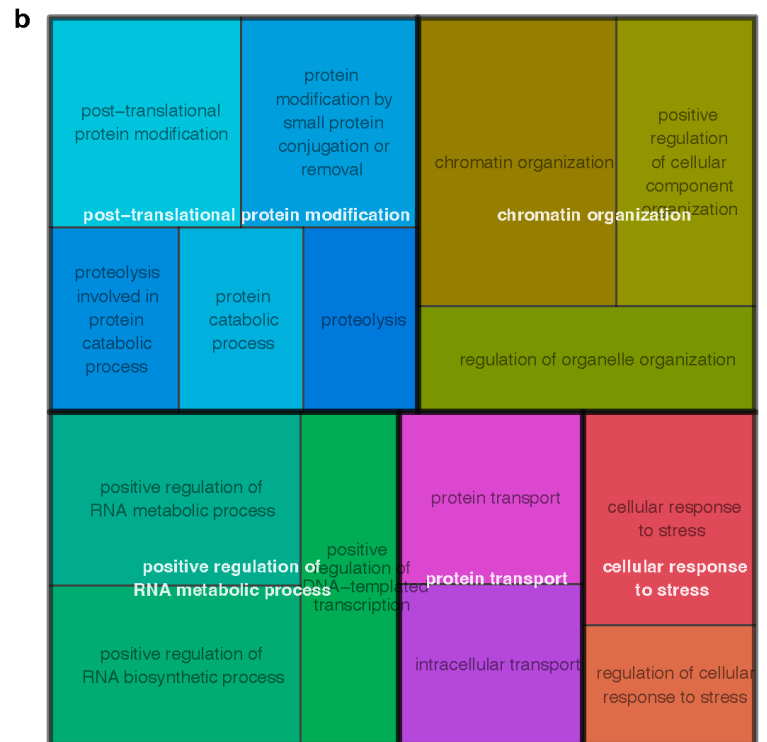
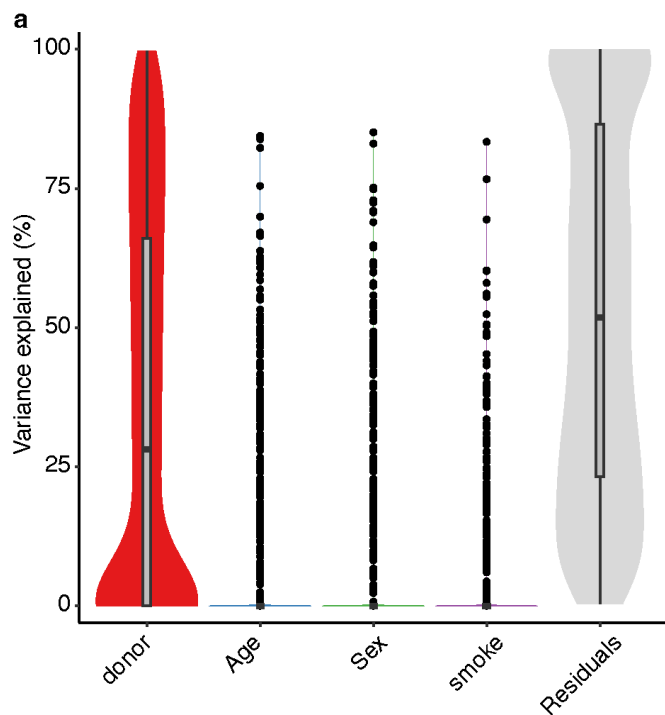
Supplementary Fig. 5: The single-cell disease relevance score (scDRS) of brain-related diseases across cells in the cortex and olfactory epithelium (OE). scDRS association between cortex cells and neuropsychiatric disorders. ADHD: Attention-deficit/hyperactivity disorder, ASD: Autism spectrum disorder, 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 $\text{fdr} < 0.05$ and $p < 0.05$, respectively.



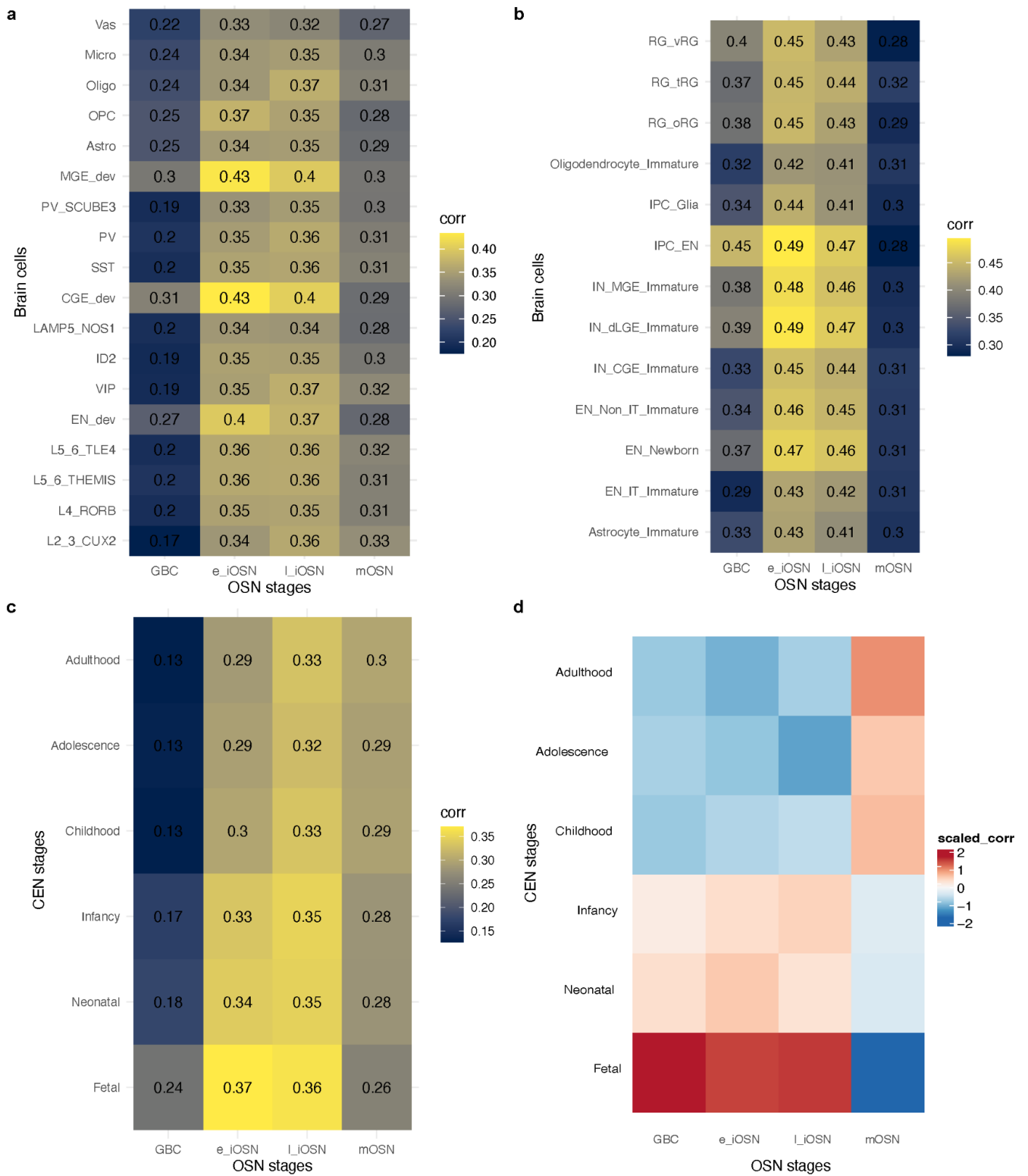
Supplementary Fig. 6: Distribution of scDRS score across datasets and donors. scDRS score between GBC/OSN and neuropsychiatric disorders. Each point represents a single cell. Box plot indicates median, interquartile range (IQR) and $1.5 \times \text{IQR}$.



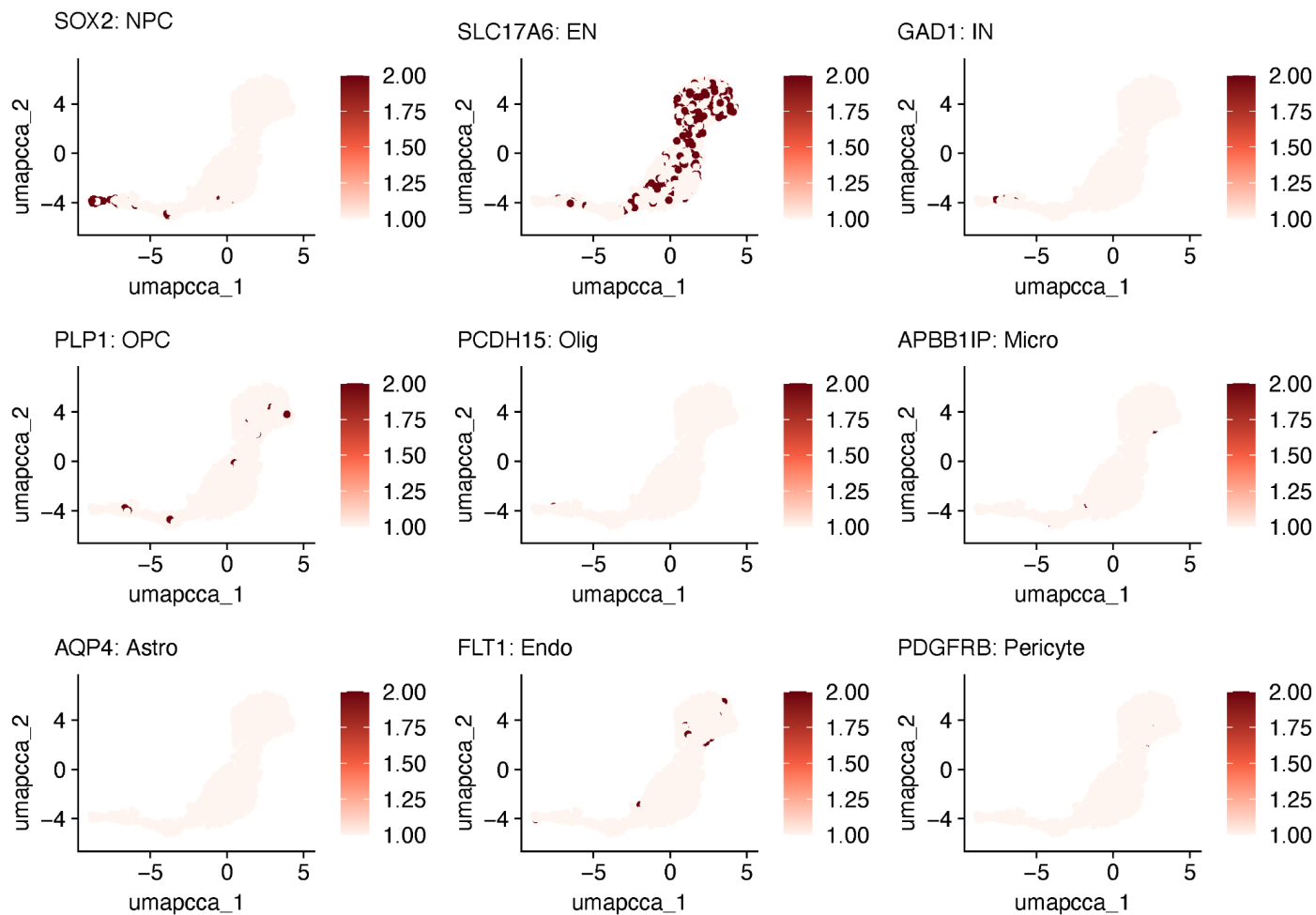
Supplementary Fig. 7: Pseudotime trajectory and differentially expressed genes along the trajectory (trajDEGs). **a**, Correlation of pseudotime assigned by the Slingshot and the Monocle3. Spearman's $R = 0.99$, $p < 2.2 \times 10^{-16}$. **b**, Overlap between trajDEGs identified by fitting a Generalized Additive Model (GAM) using the tradeSeq against slingshot pseudotime and those identified using Moran's I against monocle3 pseudotime.



Supplementary Fig. 8: 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. Box plot indicates median, interquartile range (IQR) and $1.5 \times \text{IQR}$. **b**, Functional enrichment analysis of the subset of genes with $>5\%$ of expression variance partitioned to smoking status.

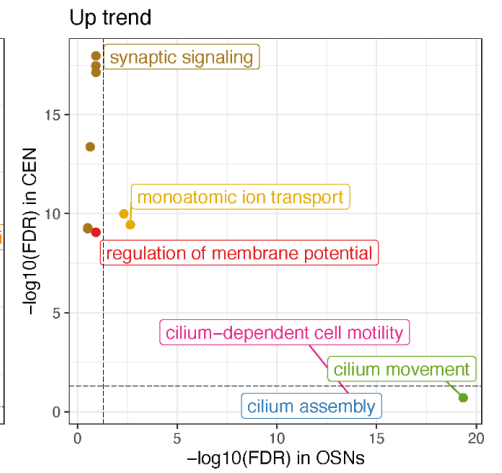
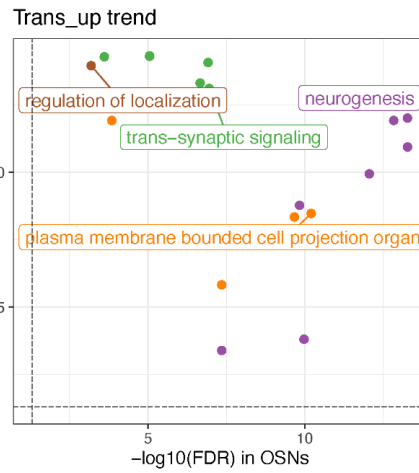
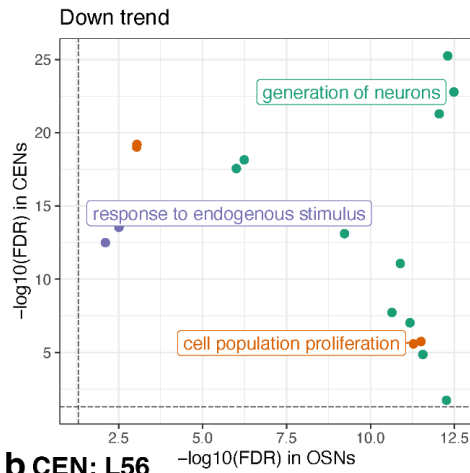


Supplementary Fig. 12: Expression similarity between GBCs/OSNs and Brain Cell Types. **a-b**, Spearman correlation of highly variable genes between GBCs/OSNs and cell types in the independent brain datasets from Herring *et al*³ (a) and Wang *et al*⁴ (b). **c**, Correlation of highly variable genes between 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.

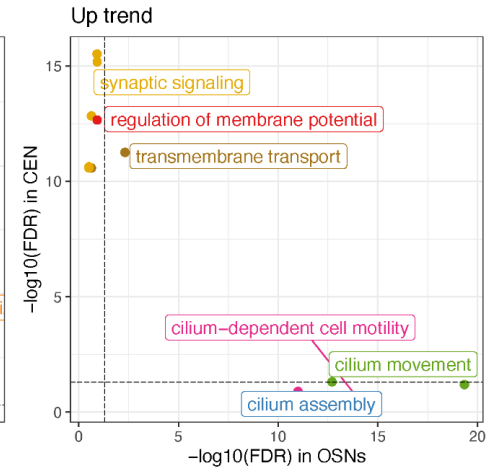
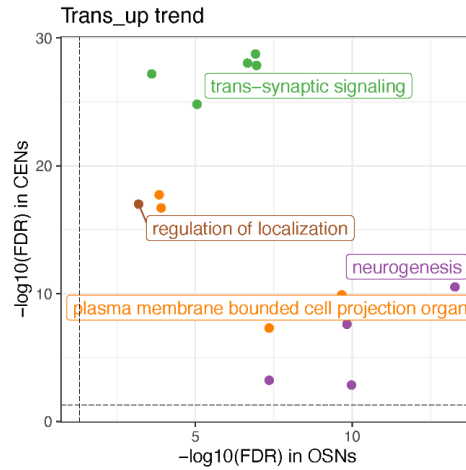
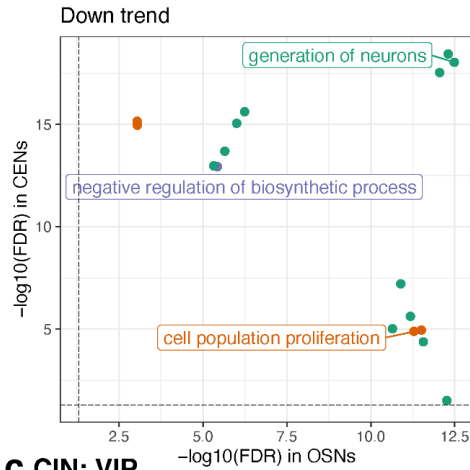


Supplementary Fig. 13: Expression of markers for brain cell types in OSNs. Cells with darker colors have higher expression levels.

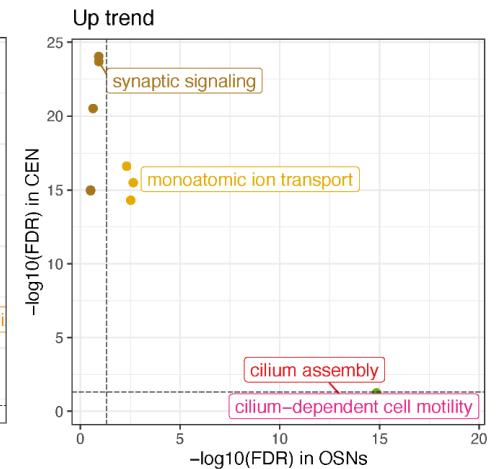
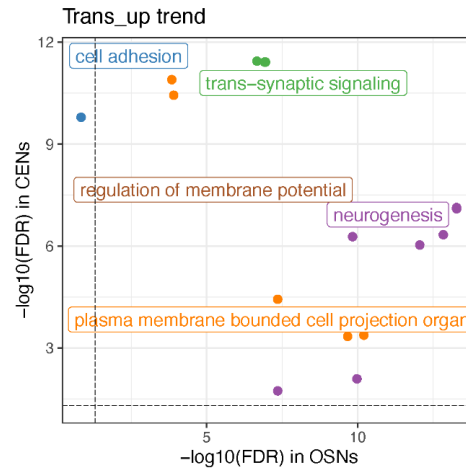
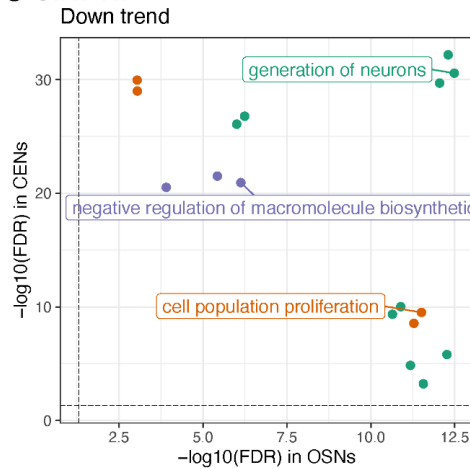
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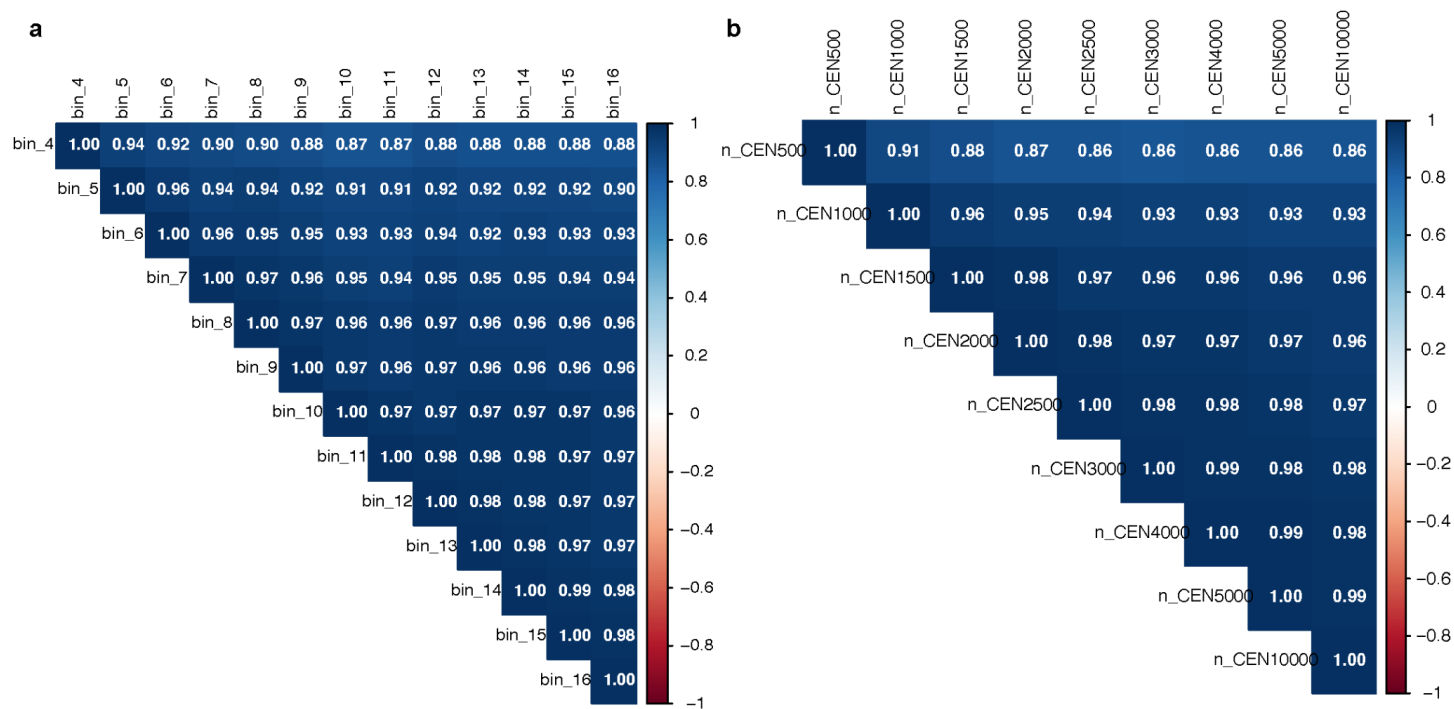
b CEN: L56



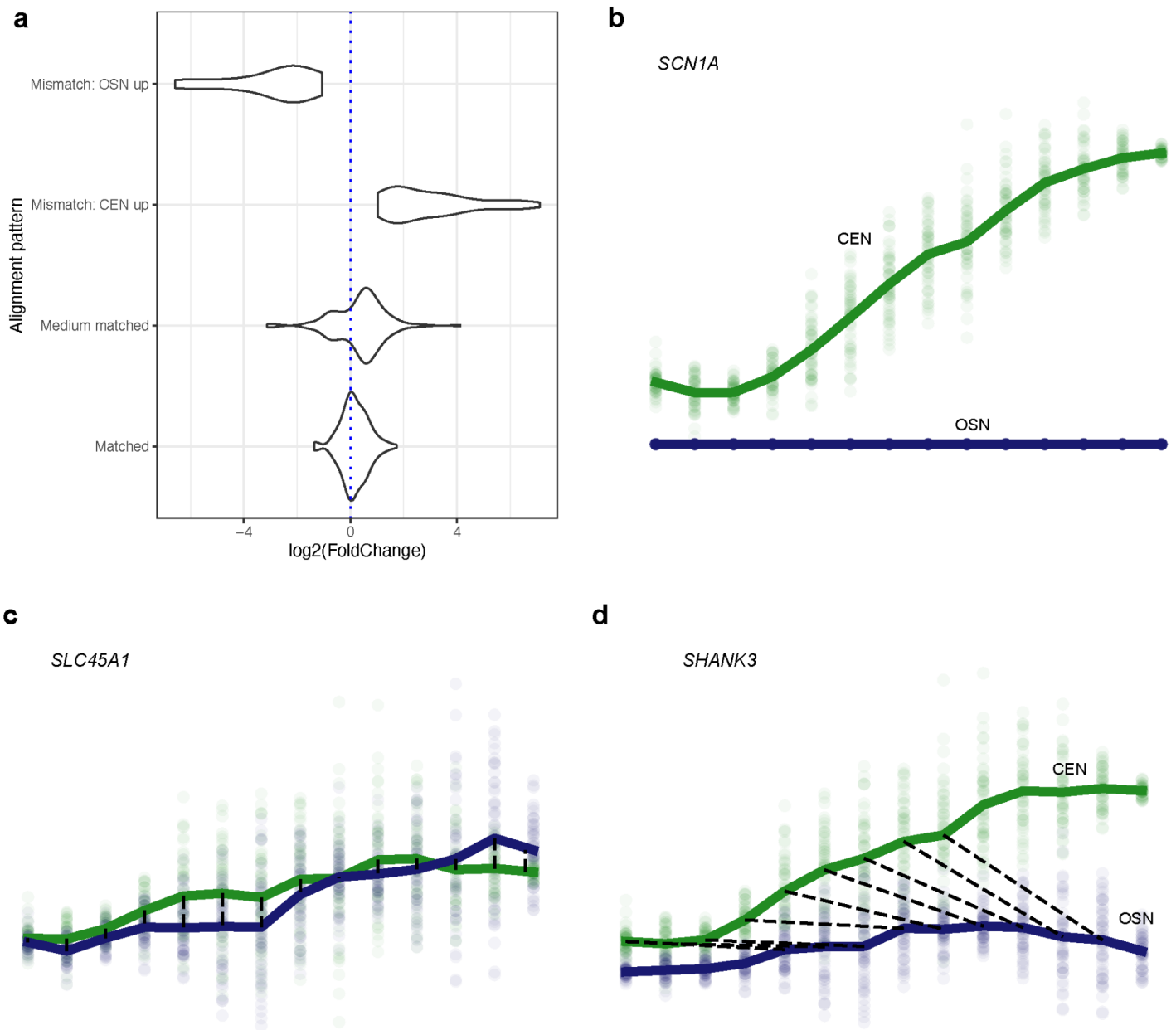
c CIN: VIP



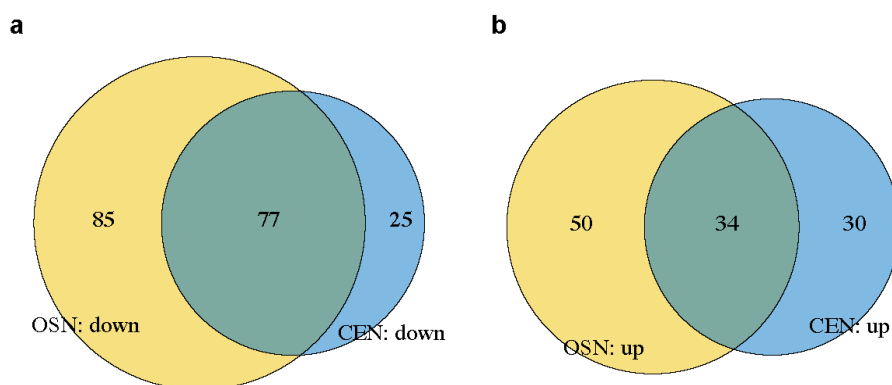
Supplementary Fig. 14: Significance of enriched biological processes for trajDEGs in OSNs compared with trajDEGs in cortical neurons. Panels a–c correspond to L4 CENs, L5/6 CENs, and VIP CINs, respectively.



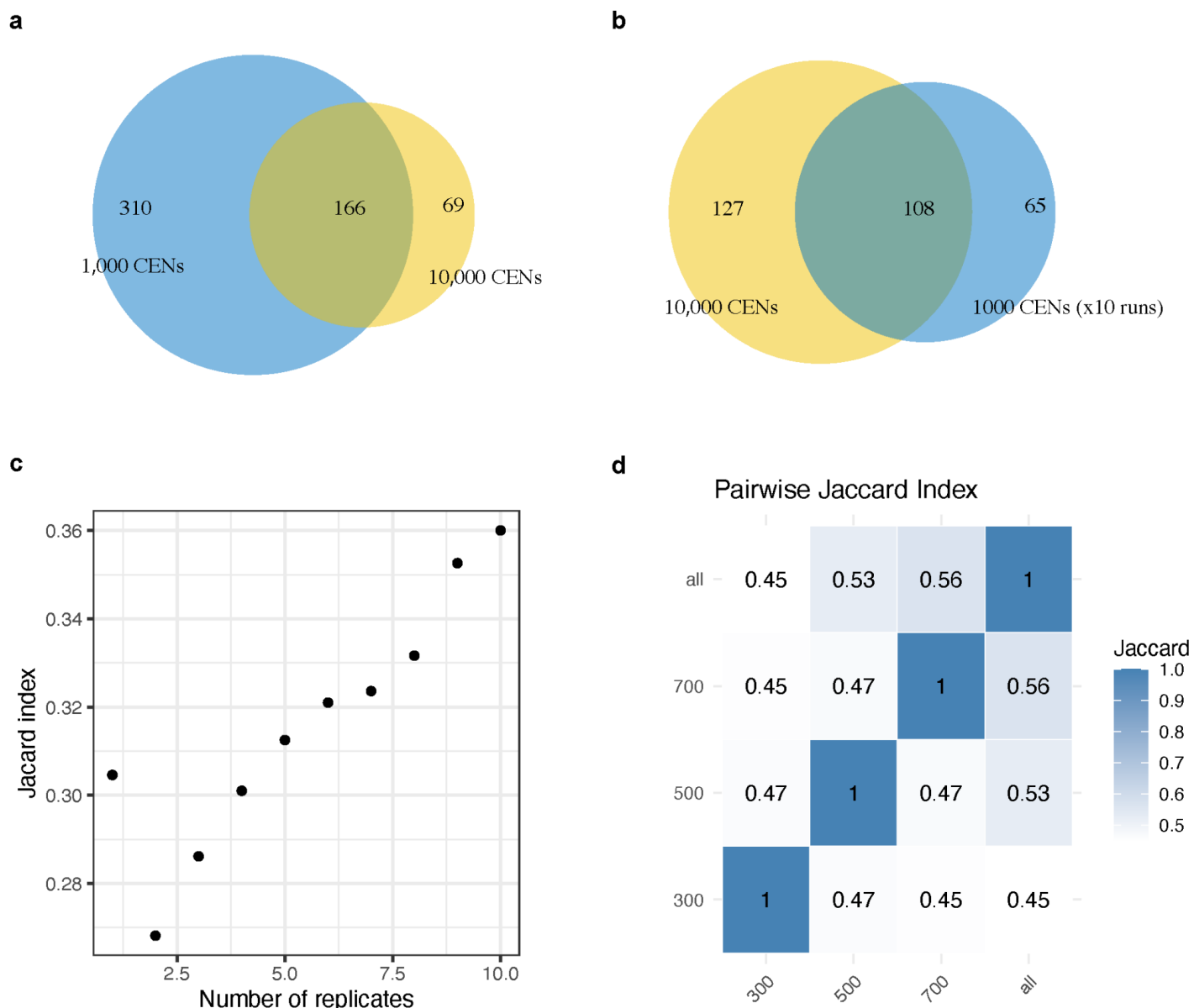
Supplementary Fig. 15: Correlation of alignment similarity percentage. (a) Correlation of alignment similarity percentage derived from varying pseudotime bin counts. (b) Correlation of alignment similarity percentages obtained by subsampling the CEN dataset to different cell counts.



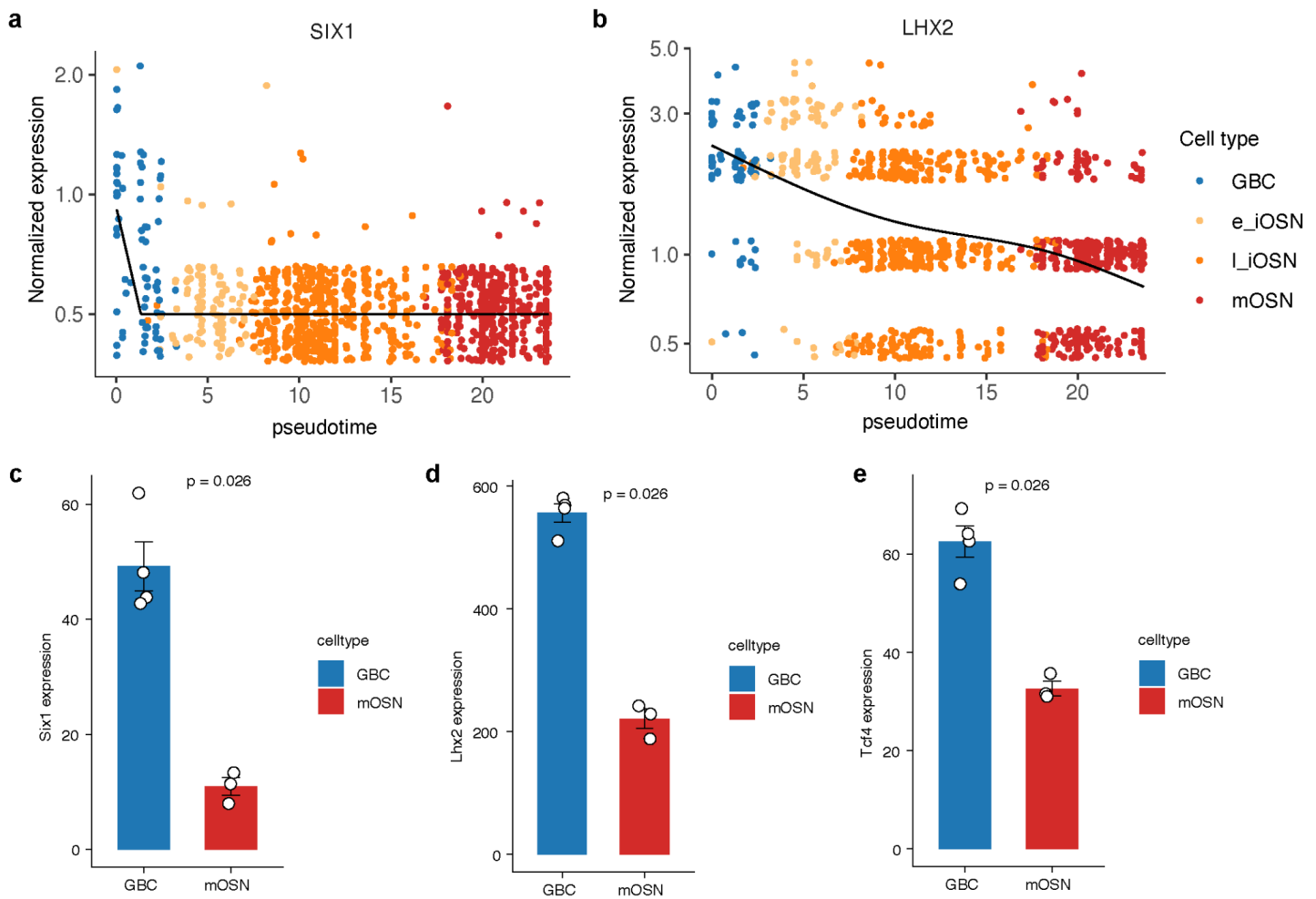
Supplementary Fig. 16: The distribution of fold changes across each alignment pattern. a. Fold changes across alignment patterns. **b-d.** Expression dynamics of *SCN1A* (**b**), *SLC45A1* (**c**), and *SHANK3* (**d**) in OSNs and CENs along pseudotime. The dashed link represents the match status between OSN and CEN at each time point.



Supplementary Fig. 17: Concordance of the dynamic trend of ASD risk genes between CENs and mouse OSNs. a, The concordance of the down-trend ASD risk genes. **b,** The concordance of the up-trend ASD risk genes.



Supplementary Fig. 18: 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.



Supplementary Fig. 19: Expression of TFs at GBCs and OSNs. a-b, The dynamic expression of *SIX1* (a) and *LHX2* (b) along pseudotime. c-e, Expression (mean $\pm$ standard error) of *Six1* (c), *Lhx2* (d), and *Tcf4* (e) across mouse GBCs and mOSNs that were purified using FACS. The significance was determined using the one-tailed Wilcoxon test.

Supplementary 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

References

1. Durante, M. A. *et al.* Single-cell analysis of olfactory neurogenesis and differentiation in adult humans. *Nat. Neurosci.* **23**, 323–326 (2020).
2. Oliva, A. D. *et al.* Aging-related olfactory loss is associated with olfactory stem cell transcriptional alterations in humans. *J. Clin. Invest.* **132**, (2022).
3. Herring, C. A. *et al.* Human prefrontal cortex gene regulatory dynamics from gestation to adulthood at single-cell resolution. *Cell* **185**, 4428–4447.e28 (2022).
4. Wang, L. *et al.* Molecular and cellular dynamics of the developing human neocortex. *Nature* 1–10 (2025).