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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	PISA (v0.2) was used to demultiplex raw sequencing reads; STAR (v2.5.1b) was used to map reads from snRNA-seq and spatial RNA-seq; sambamba (v0.7.0) was used to process bam file after alignment; SoupX (v1.4.8) was used to remove ambient RNA noise; DoubletFinder (v2.0) was used to detect the potential doublets from each individual library; Seurat (v4.0.5) was used as a tool for cell clustering and DEG identification between clusters on a high-quality dataset; DIAMOND (v0.8.23) was used to identify two-way reciprocal best hits between species; clusterProfiler (v3.18.1) was used for Gene Ontology enrichment analyses; MetaNeighbor framework (v1.10.0) was used to explore transcriptional similarity between clusters; edgeR (v3.32.1) was used to identify DEGs between different experimental groups; Tangram (v0.21.1) was used to project single-cell atlases to stereo-seq data; pySCENIC (v0.6.6) was used to predict gene regulatory network in cellular resolution. Fluorescence intensity was analyzed using ImageJ software (v1.8.0). Multiple comparisons were performed after the one-way ANOVA using the 'LSD.test' function from the 'agricolae' package (v1.3.5). The scripts used in this study have been made available on GitHub repository (https://github.com/mumu8910/snRNA_NF.git) with the identifier (https://doi.org/10.5281/zenodo.14909482)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The raw data of the snRNA-seq and the Stereo-seq have been deposited into CNGB Sequence Archive of China National GeneBank DataBase with accession number CNP0003087 and CNP0003197, respectively. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample sizes were determined based on the need to accurately capture the main cell populations of the honeybee brain, following similar sample size in previous studies (Zhang, W. et al., iScience, 2022; Traniello, I.M. et al., Nat Ecol Evol, 2023). A total of 121,247 single nuclei were analyzed for the construction of the Apis mellifera brain cell atlas, including 70,060 cells from nurses and 51,187 cells from foragers. For Stereo-seq, two cryosection slices (10 um thick) of the brains from nurse and forager bees were loaded onto the chip for profiling. Based on the 3D brain model of Apis mellifera (Rybak, J. et al., Front Syst Neurosci, 2010), the positioning of the slices ensured that key brain structures were adequately covered in our analysis, particularly the mushroom body region.
Data exclusions	For snRNA-seq, 143,228 nuclei were retained from the brain tissues. Data points with nFeature_RNA (number of expressed genes in cell) distributed from 5th to 95th percentile were retained, with each gene being detected in at least three cells. Cells with high mitochondrial content were discarded (the percentage of UMIs classified as mitochondrial genes < 0.03). Furthermore, 6382 doublets were removed for downstream analysis. Finally, we obtained a high-quality dataset containing 121,247 nuclei from 54 libraries and all libraries showed a mean of UMI > 600. In Stereo-seq, the datasets were aggregated for each section into 50x50 DNB bins (bin 50) and obtained a total of 4,741 bins with ~18,000 unique transcripts and more than 1,200 genes.

Replication	We isolated nuclei from the brains of 136 nurse and 80 forager honeybees for this study. For each biological replicate, four frozen brain tissues were used to generate one snRNA library. In total, we obtained 20 biological replicates for forager brains and 34 biological replicates for nurse brains.
Randomization	This is not relevant to our study. Honeybees were allocated into two groups based on their natural behavioral transitions.
Blinding	The investigators were blinded to group allocation during data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Worker honeybees (Apis mellifera) were used in this study. Nursing and forager bees are collected from the bee hives based on their behavior characteristics.
Wild animals	This study did not involve wild animals.
Reporting on sex	Our study was conducted on Apis mellifera workers, specifically focusing on foragers and nurses. As honeybee workers are female, only female individuals were included in all experiments. Sex was not considered as a variable in the study design.
Field-collected samples	The fieldwork was conducted in August 2020 at the apiary of China Agricultural University, Beijing, China. The study involved honeybees (Apis mellifera) of female worker. To control age, single-cohort colonies were set up, and newly emerged bees were marked and returned to their hives. Ten-day-old marked bees observed placing their heads into honeycomb cells containing eggs or larvae were collected as nurse bees. Age-matched 21- to 23-day-old bees returning to the colony with pollen loads on their legs were captured as foragers. All bees were collected between 9:00 and 11:00 a.m. on August 20, 2020. There is no current requirement regarding insect care and use in research. Honeybees were cared for daily with adequate food during the experimental period. For tissue collection, bees were collected gently and immediately euthanized by CO2 anesthesia before dissection to reduce any unnecessary duress.
Ethics oversight	No ethical approval was required in this case.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*