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Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   The sample sizes were determined based on the literatures in the fields. No statistical were used to predetermine sample sizes.

2. Data exclusions

   Describe any data exclusions.

   For developmental progression analysis, we excluded the cerebellum and olfactory bulb from analysis due to their significant morphological change during early postnatal development. We also excluded cortical layer 1 because the cell numbers of this outermost area of the brain could be influenced by the residual arachnoid matter or the possible damages during the dissection procedures.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   CUBIC-X pipeline was repeated more than ten times with independent brains and the results were reliably reproduced. For whole-brain cell counting, three brains for each age were used. The cell numbers and their variations in the areas used for early postnatal developmental analysis are shown in Figure 6 and Supplementary Figs. 20, 21. All three brains show the same trends.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   The mice used for whole-brain cell counting were randomly chosen from colonies.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   No blinding was done in this study because knowledge of experimental conditions was required during data collection. Analysis was conducted using automated workflow.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Terastitcher, MATLAB, ANTs, networkX, Gephi, Imaris, R, Graphpad Prism, Microsoft excel, LABVIEW, Image J and Fusion360 were used. We used custom scripts for the construction of CUBIC-Atlas with anatomical annotation, and for activity mapping on CUBIC-Atlas.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used .

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used .

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used .

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

6-month-old Thy1-YHP-H transgenic female mice, 10-week-old PLP-YFP (PLP-tTA::tetO-ChR2EYFP) transgenic male mice and 11-week-old Mcl1-YFP (Mlc1-tTA::tetO-YC) transgenic female mice were used to render whole-brain imaging of fluorescent reporter proteins. 4-month-old R26-H2B-EGFP male mice were used for observation of influence of delipidation over nuclei. 8-week-old male mice of C57BL/6J were used to build the whole brain atlas with single-cell resolution. C57BL/6N mice were used to analyze whole-brain cell profiling over different aged mice. 1-week-old male mice were purchased from CLEA Japan, and 3-, 8-week- and 6-month-old male mice were from Japan SLC, Inc.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The research did not involve human research participants.