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# Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

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.

   >60,000 single cells were profiled from multiple brains and brain regions to obtain reproducible overall representation of the major cell types in the juvenile zebrafish brain. Sample size for assessing lineage barcode editing using genomic DNA was chosen to demonstrate highly independent editing patterns from multiple animals.

2. Data exclusions

   Describe any data exclusions.

   Cells with fewer than 500 expressed genes, greater than 9% mitochondrial content or very high numbers of UMIs and gene counts that were outliers of a normal distribution (likely doublets/multiplets) were removed from further analysis of scRNA-seq data.

3. Replication

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

   scRNA-seq and genomic DNA libraries were prepared from multiple biological replicates. All attempts at replications were successful. Clusters identified by scRNA-seq were supported by cells from multiple biological replicates. Lineage barcode editing was successful in multiple independent embryos

4. Randomization

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

   No randomization was required for this study since no comparisons were made between samples/experimental groups

5. Blinding

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

   No blinding was required for this study since no comparisons were made between samples/experimental groups

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<br>*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<br>*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.

Bowtie1 was used for scRNA-seq alignments. Trimmomatic and PHYLIP packages were used for processing. MAFFT and NEEDLEALL aligners were used for aligment. Monocle 2 was used for differentiation trajectory analysis. Seurat v1.4 was used for clustering analysis. ApE (v2.0.50b3) was used for large deletion alignments. D3 software was used for tree visualization. R(v3.4.0) and Rstudio (v1.0.143) were used for data analysis. Custom data processing pipelines are available at: https://github.com/aaronmck/SC_GESTALT and https://github.com/indrops/indrops

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.

There are no restrictions to materials availability

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 in this study

10. Eukaryotic cell lines

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

No eukaryotic cell lines were used in this study

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used in this study

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

No eukaryotic cell lines were used in this study

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 eukaryotic cell lines were used in this study

## 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.

Danio rerio. TL/AB strain. Embryos (2dpf) and juvenile animals (23-25dpf) were used. Sex indeterminate at these stages.

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.

This study did not involve human research participants