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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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.

Experimental design

1. Sample size

Describe how sample size was determined.

This is a first in kind study imaging Ca2+ activity in myelin sheaths in vivo using zebrafish. Therefore, it was not possible to do power calculations prior to the study, as the nature of the signalling and its variability was unknown. For these Ca2 + imaging experiments we imaged 18 animals and a total of 305 myelin sheaths, 187 of which showed activity. Analysis of variation indicated diverse Ca2+ activity between individual sheaths, and so "n" in the Ca2+ imaging experiments relates to individual sheaths. For our analyses of how manipulations of calpain signalling (chemical and genetic) affect myelination by individual oligodendrocytes, we carried out power calculations, and all experiments documented in Figure 2 and Supplementary 6 had a power >80%.

2. Data exclusions

Describe any data exclusions.

No data were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All findings were reproduced in at least two different experimental replicates. The details of experimental replicates are indicated in the appropriate figure legends.

4. Randomization

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

No randomisation was carried out on animals whose myelin sheaths were Ca2+ imaged, as all are wildtype- no experimental manipulation. For chemical and genetic manipulations, zebrafish embryos for both experimental and control conditions derived from the same clutch (per experiment). For time-lapse analyses of chemical inhibitor treated animals, control and experimental animals were imaged separately for technical reasons. Embryos were grown up in the same incubator and the same conditions prior to analyses, all live imaging. During live imaging analyses, experimental and control animals were imaged in an alternating pattern (per experiment) to ensure no confounding effects of stage of development between groups.

5. Blinding

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

The analysis of all experimental findings were carried out blinded and image data was randomised using a custom-made script.

Note: all studies involving animals and/or human research participants must disclose 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals 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

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.	Image acquisition: iQ2 and iQ3 (Andor), Zen (Zeiss). Analysis: Fiji version 1.0, ImageJ 1.50c4, Microsoft Excel version 15.32, Clampfit version 10.7.0.3, Patch clamp (Molecular device), SnapGene viewer version 3.1.2, CLC Main Workbench 7 (Qiagen), Graph pad (Prism6 and 7 and StatMate). For figure preparation: Matlab R2015b (to make graph), Adobe Illustrator CS6.

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 for-profit company.	All transgenic zebrafish, transgenic constructs and relevant information will be made available upon request.

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

Zebrafish (danio rerio) were used in this study. This included a mix of stable transgenic lines, a mutant line, and transient transgenic animals generated by injection of transgenes at the one cell stage. The stable transgenic lines that were used were tg(sox10(7.2):KalTA4GI),tg(UAS:mem-GFP),tg(uas:GCaMP6s) and tg(sox10:mRFP). We used the nacre mutant (which lacks melanophores). Transient transgenic animals were injected with the following constructs: mbp:mCherry-CAAX, mbp:meGFP, mbp:meGFP_calpastatin, and calpastatin mRNA. Adult zebrafish, up to two years of age, were used for breeding purposes. All experimental analyses in the manuscript were carried out on animals up to 5 days post fertilisation. Details of stages used for individual experiments is detailed in the main text and or figures and legends.

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