

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Raw immunofluorescence images were captured and stitched using Zeiss ZEN (v3.5). 3D reconstruction images and movies were generated using Imaris 9.0 (Bitplane).
Data analysis	Computer codes associated with the manuscript are available on the JLiLab GitHub: https://github.com/JLiLab/Studying-Parkinje-cell-heterogeneity . We additionally used Seurat (v4.3.0 and v5.1.0), fastMNN from batchelor (v1.18.1), clusterProfiler (v4.10.0), and COSG (v 0.9.0). Sequencing reads of scRNA-seq of mouse cerebella were demultiplexed and aligned to the mm10 mouse genome reference using Cell Ranger v3.1. Sequencing reads of scRNA-seq chick cerebella were demultiplexed using split-pipe v1.0.6p and aligned to the chicken genome GRCg7b and Ensembl Release 110 annotations. The 'split-pipe' pipeline was used to generate a cell-by-gene count matrix.

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](#)

Sequencing data are publicly available in GEO under accession codes GSE256438 and GSE278923. The publicly available datasets were used in this study: GSE118068 and PRJNA637987. BAM files of published data were obtained from the European Nucleotide Archive (PRJNA637987) and NCBI GEO (GSE118068) and then converted to FASTQ using bamtofastq (v1.3.2).

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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](https://www.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	No statistical methods were used to predetermine sample sizes. Sample sizes reflect those commonly used in the field and our previous studies 13,58.
Data exclusions	No data or animals were excluded from this study.
Replication	All attempts at replication were successful and noted in the figures/text of paper. Detailed information is described in the methods section under "statistics and reproducibility".
Randomization	Animals were assigned to experimental groups based on genotype. No additional randomization or blocking was used during data collection or analysis. Experimental conditions and sample processing were conducted in parallel where possible to minimize batch effects.
Blinding	Data collection and analysis were not performed blind to experimental conditions. However, quantification was conducted in an unbiased manner using either automated image analysis or by an operator blinded to genotype.

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

Methods

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

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

Antibodies

Antibodies used

Primary Aldoc Goat 1/200 Santa Cruz Biotech, Inc. sc-12065 AB_2242641
 Primary Bcl11a Mouse 1/500 Santa Cruz Biotech, Inc. sc-514842 n/a
 Primary Bcl11b Rat 1/1000 abcam ab18465 AB_2064130
 Primary Calb1 Mouse 1/4000 SIGMA C9848 AB_476894
 Primary Calb1 Rabbit 1/3000 Swant CB-38a AB_3107026
 Primary Calb2 Rabbit 1/1000 Swant 7699/4 AB_2313763
 Primary Dab1 Mouse 1/100 Santa Cruz Biotech, Inc. sc-271136 AB_10610240
 Primary Ebf Mouse 1/2000 Santa Cruz Biotech, Inc. sc-137065 AB_2246405
 Primary En1 Rabbit 1/400 Gift from Dr. Alex Joyner n/a n/a
 Primary Eomes Rat 1/1000 ThermoFisher Scientific 14-4875-82 AB_11042577
 Primary Etv1 Rabbit 1/400 Gift from Dr. Silvia Arber n/a n/a
 Primary Fabp7 Guinea pig 1/1000 Nittobo Medical MSFR100320 n/a
 Primary Foxp1 Mouse 1/400 Santa Cruz Biotech, Inc. sc-398811 n/a
 Primary Foxp1 Guinea pig 1/16,000 Gift from Dr. Bennett Novitch n/a n/a
 Primary Foxp2 Mouse 1/200 Santa Cruz Biotech, Inc. sc-517261 AB_2721204
 Primary Foxp2 Goat 1/4000 ARP American Research Products, Inc EB05226 AB_2107112
 Primary Foxp4 Rabbit 1/1000 Proteintech 16772-1-AP AB_2262825
 Primary Hspb1 Rabbit 1/500 Enzo Life Sciences, Inc ADI-SPA-801 AB_10615795
 Primary Isl1 Mouse 1/100 DSH Bank 39.4D5 AB_2314683
 Primary Lhx1/5 Mouse 1/100 DSH Bank 4F2 AB_531784
 Primary Meis2 Mouse 1/200 Santa Cruz Biotech, Inc. sc-515470 AB_3076386
 Primary Mki67 Rat 1/1000 Invitrogen 14-5698-82 AB_10854564
 Primary Neurod1 Mouse 1/100 Santa Cruz Biotech, Inc. sc-46684 AB_671759
 Primary Neurod2 Mouse 1/200 Santa Cruz Biotech, Inc. sc-365896 AB_10843361
 Primary Nr2f2 Mouse 1/2000 abcam AB41859 AB_742211
 Primary Nrgn Rabbit 1/800 Millipore AB5620 AB_91937
 Primary Olig2 Goat 1/200 Santa Cruz Biotech, Inc. sc-19969 AB_2236477
 Primary Olig2 Rabbit 1/500 abcam ab109186 AB_10861310
 Primary Otx2 Goat 1/400 R&D Systems, Inc. AF1979 AB_2157172
 Primary Pax2 Rabbit 1/500 Invitrogen 71-6000 AB_2533990
 Primary Pax3 Mouse 1/100 DSH Bank Pax3-c AB_528426
 Primary Pax5 Mouse 1/200 Santa Cruz Biotech, Inc. sc13146 AB_628106
 Primary Pax6 Rabbit 1/200 Covance PRB-278P AB_291612
 Primary Pax6 Mouse 1/300 Santa Cruz Biotech, Inc. sc-81649 AB_1127044
 Primary Pcdh10 Rat 1/1000 EMD Millipore MABT20 AB_10807416
 Primary Pcp2 Mouse 1/200 Santa Cruz Biotech, Inc. sc-137064 AB_2158439
 Primary Plcb4 Mouse 1/400 Santa Cruz Biotech, Inc. sc-166131 AB_2237003
 Primary Reln Goat 1/200 Fisher AF3820 AB_2253745
 Primary Rorb Mouse 1/300 R&D Systems, Inc. PP-N7927-00 AB_1964364
 Primary Satb2 Rabbit 1/1000 abcam ab34735 AB_2301417
 Primary Sox10 Goat 1/100 R&D Systems, Inc. AF2864-SP AB_442208
 Primary Sox14 Rabbit 1/3000 Gift from Dr. Tom Jessel n/a n/a
 Primary Sox2 Goat 1/300 R&D Systems, Inc. AF2018 AB_355110
 Primary Sox8 Mouse 1/200 Santa Cruz Biotech, Inc. sc-374446 AB_10989367
 Primary Sox9 Rabbit 1/1000 Millipore AB5535 AB_2239761
 Primary Tbr1 Rabbit 1/2000 abcam ab31940 AB_2200219
 Primary Tfp2a Mouse 1/100 Santa Cruz Biotech, Inc. sc-25343 AB_667766
 Primary Tfp2a Mouse 1/400 Santa Cruz Biotech, Inc. sc-12726 AB_667767
 Primary Tfp2b Mouse 1/200 Santa Cruz Biotech, Inc. sc-390119 AB_2828008
 Primary Tle1 Mouse 1/100 Santa Cruz Biotech, Inc. sc-137098 AB_2303387
 Primary Tlx3 Rabbit 1/5000 Gift from Dr. Thomas Müller n/a n/a
 Primary Tlx3 Guinea pig 1/10,000 Gift from Dr. Thomas Müller n/a n/a
 Primary Zic1 Goat 1/1000 R&D Systems, Inc. AF4978 AB_2219353
 Secondary Chicken IgY, Alexa 488 Donkey 1/1000 Jackson ImmunoResearch 703-545-155
 Secondary Chicken IgY, Alexa 594 Donkey 1/1000 Jackson ImmunoResearch 703-586-155
 Secondary Goat IgG (H+L), Alexa 488 Donkey 1/1000 Jackson ImmunoResearch 705-545-147
 Secondary Goat IgG (H+L), Alexa 546 Donkey 1/1000 Invitrogen A-11056
 Secondary Goat IgG (H+L), Alexa 647 Donkey 1/1000 Life Technologies A-21447

Secondary Goat IgG (H+L), Alexa 594 Donkey 1/1000 Molecular Probes A-11058
 Secondary Goat IgG (H+L), Alexa 750 Donkey 1/1000 abcam ab175744
 Secondary Guinea Pig IgG (H+L), Alexa 488 Donkey 1/1000 Jackson ImmunoResearch 705-545-148
 Secondary Guinea Pig IgG (H+L), Alexa 594 Donkey 1/1000 Jackson ImmunoResearch 706-585-148
 Secondary Guinea Pig IgG(H+L), Alexa 647 Donkey 1/1000 Jackson ImmunoResearch 706-605-148
 Secondary Mouse IgG (H+L), CF*430 Donkey 1/1000 Biotium 20461-500µl
 Secondary Mouse IgG (H+L), Alexa 488 Donkey 1/1000 ThermoFisher Scientific A21202
 Secondary Mouse IgG (H+L), Alexa 546 Donkey 1/1000 ThermoFisher Scientific A10036
 Secondary Mouse IgG(H&L), Alexa 750 Donkey 1/1000 abcam ab175737
 Secondary Mouse IgG1, CF*543 Goat 1/1000 Biotium 20235
 Secondary Mouse IgG1, CF*647 Goat 1/1000 Biotium 20252
 Secondary Mouse IgG1, CF*488A Goat 1/1000 Biotium 20246
 Secondary Mouse IgG2a, CF*647 Goat 1/1000 Biotium 20262
 Secondary Mouse IgG2a, CF*488A Goat 1/1000 Biotium 20256
 Secondary Mouse IgG2a, CF*543 Goat 1/1000 Biotium 20326
 Secondary Mouse IgG2b, CF*594 Goat 1/1000 Biotium 20269
 Secondary Mouse IgG2b, CF*750 Goat 1/1000 Biotium 20430
 Secondary Mouse IgG2b, CF*488A Goat 1/1000 Biotium 20266
 Secondary Mouse IgG2b, CF*647 Goat 1/1000 Biotium 20272
 Secondary Mouse IgM, Alexa488 Donkey 1/1000 Jackson ImmunoResearch 715-545-020
 Secondary Mouse IgM, Alexa 647 Donkey 1/1000 Jackson ImmunoResearch 715-605-020
 Secondary Rabbit IgG, Alexa 488 Donkey 1/1000 Invitrogen A21206
 Secondary Rabbit IgG (H+L), Alexa 546 Donkey 1/1000 ThermoFisher Scientific A10040
 Secondary Rabbit IgG (H+L), Alexa 647 Donkey 1/1000 Invitrogen A-31573
 Secondary Rabbit IgG (H+L), Alexa 594 Donkey 1/1000 ThermoFisher Scientific A-21207
 Secondary Rabbit IgG H&L, Alexa 750 Donkey 1/1000 abcam ab175731
 Secondary Rabbit IgG(H+L), CF*430 Donkey 1/1000 Biotium 20462
 Secondary Rat IgG, Alexa 488 Donkey 1/1000 Invitrogen A-21208
 Secondary Rat IgG, CF*750 Donkey 1/1000 Biotium 20857
 Secondary Rat IgG, Alexa 594 Donkey 1/1000 Invitrogen A21209
 Secondary Rat IgG, CF*647 Donkey 1/1000 Biotium 20843

Validation

Most antibodies used in this study are commercially available and have been validated by the manufacturer.

Antibody Antigen Source Catalog # Link for Validation

Primary Aldoc Santa Cruz Biotech, Inc. sc-12065 <https://www.scbt.com/p/aldolase-c-antibody-n-14>

Primary Bcl11a Santa Cruz Biotech, Inc. sc-514842 <https://www.scbt.com/p/bcl-11a-antibody-h-1>

Primary Bcl11b abcam ab18465 <https://www.abcam.com/en-us/products/primary-antibodies/ctip2-antibody-25b6-ab18465>

Primary Calb1 SIGMA C9848 <https://www.sigmaaldrich.com/US/en/product/sigma/c9848>

Primary Calb1 Swant CB-38a <https://webshop.swant.com/cb38a-calbindin.html>

Primary Calb2 Swant 7699/4 <https://webshop.swant.com/cb300-calbindin-1.html>

Primary Dab1 Santa Cruz Biotech, Inc. sc-271136 <https://www.scbt.com/p/dab1-antibody-g-5>

Primary Ebf Santa Cruz Biotech, Inc. sc-137065 <https://www.scbt.com/p/ebf-antibody-c-8>

Primary En1 Gift from Dr. Alex Joyner n/a n/a

Primary Eomes ThermoFisher Scientific 14-4875-82 <https://www.thermofisher.com/antibody/product/EOMES-Antibody-clone-Dan11mag-Monoclonal/14-4875-82>

Primary Etv1 Gift from Dr. Silvia Arber n/a n/a

Primary Fabp7 Nittobo Medical MSFR100320 chrome-extension://efaidnbmninnibpcjpcglclefindmkaj/<https://nittobo-nmd.co.jp/pdf/reagents/BLBP.pdf>

Primary Foxp1 Santa Cruz Biotech, Inc. sc-398811 <https://www.scbt.com/p/foxp1-antibody-a-2>

Primary Foxp1 Gift from Dr. Bennett Novitch n/a n/a

Primary Foxp2 Santa Cruz Biotech, Inc. sc-517261 <https://www.scbt.com/p/foxp2-antibody-5c11a8?search-input=sc-517261>

Primary Foxp2 ARP American Research Products, Inc EB05226 <https://everestbiotech.com/product/goat-anti-foxp2-c-terminus-antibody/>

Primary Foxp4 Proteintech 16772-1-AP <https://www.ptglab.com/results?q=16772-1-AP>

Primary Hspb1 Enzo Life Sciences, Inc ADI-SPA-801 <https://www.enzo.com/product/hsp25-polyclonal-antibody/>

Primary Isl1 DSH Bank 39.4D5 <https://dshb.biology.uiowa.edu/39-4D5>

Primary Lhx1/5 DSH Bank 4F2 <https://dshb.biology.uiowa.edu/4F2>

Primary Meis2 Santa Cruz Biotech, Inc. sc-515470 <https://www.scbt.com/p/meis2-antibody-h-10?search-input=sc-515470>

Primary Mki67 Invitrogen 14-5698-82 <https://www.thermofisher.com/antibody/product/Ki-67-Antibody-clone-SolA15-Monoclonal/14-5698-82>

Primary Neurod1 Santa Cruz Biotech, Inc. sc-46684 <https://www.scbt.com/p/neuro-d-antibody-a-10?search-input=sc-46684>

Primary Neurod2 Santa Cruz Biotech, Inc. sc-365896 <https://www.scbt.com/p/neuro-d-antibody-a-10?search-input=sc-365896>

Primary Nr2f2 abcam AB41859 <https://www.abcam.com/en-us/products/primary-antibodies/nr2f2-antibody-h7147-ab41859>

Primary Nrgn Millipore AB5620 <https://www.sigmaaldrich.com/US/en/product/mm/ab5620>

Primary Olig2 Santa Cruz Biotech, Inc. sc-19969 <https://www.scbt.com/p/olig2-antibody-c-17?search-input=sc-19969>

Primary Olig2 abcam ab109186 <https://www.abcam.com/en-us/products/primary-antibodies/olig2-antibody-epr2673-ab109186>

Primary Otx2 R&D Systems, Inc. AF1979 https://www.rndsystems.com/products/human-otx2-antibody_af1979

Primary Pax2 Invitrogen 71-6000 <https://www.thermofisher.com/antibody/product/PAX2-Antibody-Polyclonal/71-6000>

Primary Pax3 DSH Bank Pax3-c <https://dshb.biology.uiowa.edu/product/3929>

Primary Pax5 Santa Cruz Biotech, Inc. sc13146 <https://www.scbt.com/p/pax-5-antibody-a-11?search-input=sc13146>

Primary Pax6 Covance PRB-278P <https://www.biolegend.com/en-gb/products/purified-anti-pax-6-antibody-11511>

Primary Pax6 Santa Cruz Biotech, Inc. sc-81649 <https://www.scbt.com/p/pax-6-antibody-pax6?search-input=sc-81649>

Primary Pcdh10 EMD Millipore MABT20 <https://www.sigmaaldrich.com/US/en/product/mm/mabt20>

Primary Pcp2 Santa Cruz Biotech, Inc. sc-137064 <https://www.scbt.com/p/pcp-2-antibody-f-3?search-input=sc-137064>

Primary Plcb4 Santa Cruz Biotech, Inc. sc-166131 <https://www.scbt.com/p/plc-beta4-antibody-a-8?search-input=sc-166131>

Primary Reln Fisher AF3820 https://www.rndsystems.com/products/mouse-reelin-antibody_af3820

Primary Rorb R&D Systems, Inc. PP-N7927-00 https://www.rndsystems.com/products/human-ror-beta-antibody-clone-n7927-n7927_pp-n7927-00

Primary Satb2 abcam ab34735 <https://www.abcam.com/en-us/products/primary-antibodies/satb2-antibody-ab34735>

Primary Sox10 R&D Systems, Inc. AF2864-SP https://www.rndsystems.com/products/human-sox10-antibody_af2864

Primary Sox14 Gift from Dr. Tom Jessel n/a n/a

Primary Sox2 R&D Systems, Inc. AF2018 https://www.rndsystems.com/products/human-mouse-rat-sox2-antibody_af2018

Primary Sox8 Santa Cruz Biotech, Inc. sc-374446 <https://www.scbt.com/p/sox-8-antibody-h-7?search-input=sc-374446>

Primary Sox9 Millipore AB5535 <https://www.sigmaaldrich.com/US/en/product/mm/ab5535>

Primary Tbr1 abcam ab31940 <https://www.abcam.com/en-us/products/primary-antibodies/tbr1-antibody-ab31940>

Primary Tfpap2a Santa Cruz Biotech, Inc. sc-25343 <https://www.scbt.com/p/ap-2alpha-antibody-d-12?search-input=sc-25343>

Primary Tfpap2a Santa Cruz Biotech, Inc. sc-12726 <https://www.scbt.com/p/ap-2alpha-antibody-3b5?search-input=sc-12726>

Primary Tfpap2b Santa Cruz Biotech, Inc. sc-390119 <https://www.scbt.com/p/ap-2beta-antibody-c-6?search-input=sc-390119>

Primary Tle1 Santa Cruz Biotech, Inc. sc-137098 <https://www.scbt.com/p/tle1-antibody-f-4?search-input=sc-137098>

Primary Tlx3 Gift from Dr. Thomas Müller n/a n/a

Primary Tlx3 Gift from Dr. Thomas Müller n/a n/a

Primary Zic1 R&D Systems, Inc. AF4978 https://www.rndsystems.com/products/human-mouse-zic1-antibody_af4978

Secondary Chicken IgY, Alexa 488 Jackson ImmunoResearch 703-545-155 <https://www.jacksonimmuno.com/catalog/products/703-545-155>

Secondary Chicken IgY, Alexa 594 Jackson ImmunoResearch 703-586-155 <https://www.jacksonimmuno.com/catalog/products/703-586-155>

Secondary Goat IgG (H+L), Alexa 488 Jackson ImmunoResearch 705-545-147 <https://www.jacksonimmuno.com/catalog/products/705-545-147>

Secondary Goat IgG (H+L), Alexa 546 Invitrogen A-11056 <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11056>

Secondary Goat IgG (H+L), Alexa 647 Life Technologies A-21447 <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21447>

Secondary Goat IgG (H+L), Alexa 594 Molecular Probes A-11058 <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11058>

Secondary Goat IgG (H+L), Alexa 750 abcam ab175744 <https://www.abcam.com/en-us/products/secondary-antibodies/donkey-goat-igg-h-l-alexa-fluor-750-ab175744>

Secondary Guinea Pig IgG (H+L), Alexa 488 Jackson ImmunoResearch 705-545-148 <https://www.jacksonimmuno.com/catalog/products/706-545-148>

Secondary Guinea Pig IgG (H+L), Alexa 594 Jackson ImmunoResearch 706-585-148 <https://www.jacksonimmuno.com/catalog/products/706-585-148>

Secondary Guinea Pig IgG(H+L), Alexa 647 Jackson ImmunoResearch 706-605-148 <https://www.jacksonimmuno.com/catalog/products/706-605-148>

Secondary Mouse IgG (H+L), CF®430 Biotium 20461-500µl https://biotium.com/product/donkey-anti-mouse-igg-hl-highly-cross-adsorbed/?attribute_pa_conjugation=cf430

Secondary Mouse IgG (H+L), Alexa 488 ThermoFisher Scientific A21202 <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202>

Secondary Mouse IgG (H+L), Alexa 546 ThermoFisher Scientific A10036 <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10036>

Secondary Mouse IgG(H&L), Alexa 750 abcam ab175737 <https://www.abcam.com/en-us/products/secondary-antibodies/donkey-fab2-mouse-igg-h-l-alexa-fluor-750-ab175737>

Secondary Mouse IgG1, CF®543 Biotium 20235 <https://biotium.com/product/goat-anti-mouse-igg1/>

Secondary Mouse IgG1, CF®647 Biotium 20252 <https://biotium.com/product/goat-anti-mouse-igg1/>

Secondary Mouse IgG1, CF®488A Biotium 20246 <https://biotium.com/product/goat-anti-mouse-igg1/>

Secondary Mouse IgG2a, CF®647 Biotium 20262 <https://biotium.com/product/goat-anti-mouse-igg2a-2a/>

Secondary Mouse IgG2a, CF®488A Biotium 20256 <https://biotium.com/product/goat-anti-mouse-igg2a-2a/>

Secondary Mouse IgG2a, CF®543 Biotium 20326 <https://biotium.com/product/goat-anti-mouse-igg2a-2a/>

Secondary Mouse IgG2b, CF®594 Biotium 20269 <https://biotium.com/product/goat-anti-mouse-igg2b-2b/>

Secondary Mouse IgG2b, CF®750 Biotium 20430 <https://biotium.com/product/goat-anti-mouse-igg2b-2b/>

Secondary Mouse IgG2b, CF®488A Biotium 20266 <https://biotium.com/product/goat-anti-mouse-igg2b-2b/>

Secondary Mouse IgG2b, CF®647 Biotium 20272 <https://biotium.com/product/goat-anti-mouse-igg2b-2b/>

Secondary Mouse IgM, Alexa488 Jackson ImmunoResearch 715-545-020 <https://www.jacksonimmuno.com/catalog/products/715-545-020>

Secondary Mouse IgM, Alexa 647 Jackson ImmunoResearch 715-605-020 <https://www.jacksonimmuno.com/catalog/products/715-605-020>

Secondary Rabbit IgG, Alexa 488 Invitrogen A21206 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206>

Secondary Rabbit IgG (H+L), Alexa 546 ThermoFisher Scientific A10040 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10040>

Secondary Rabbit IgG (H+L), Alexa 647 Invitrogen A-31573 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573>

Secondary Rabbit IgG (H+L), Alexa 594 ThermoFisher Scientific A-21207 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21207>

Secondary Rabbit IgG H&L, Alexa 750 abcam ab175731 <https://www.abcam.com/en-us/products/secondary-antibodies/donkey-rabbit-igg-h-l-alexa-fluor-750-ab175731>

Secondary Rabbit IgG(H+L), CF®430 Biotium 20462 <https://biotium.com/product/donkey-anti-rabbit-igg-hl-highly-cross-adsorbed/>

Secondary Rat IgG, Alexa 488 Invitrogen A-21208 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208>

Secondary Rat IgG, CF®750 Biotium 20857 <https://biotium.com/product/donkey-anti-rat-igg-hl-highly-cross-adsorbed/>

Secondary Rat IgG, Alexa 594 Invitrogen A21209 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21209>

Secondary Rat IgG, CF®647 Biotium 20843 <https://biotium.com/product/donkey-anti-rat-igg-hl-highly-cross-adsorbed/>

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	Mice were housed on a 12-hour light/dark cycle with ad libitum access to food and water. Genetically modified mouse strains, including Atoh1-Cre 29, En1-Cre 48, Foxp1-floxed 49, and Foxp2-floxed 23, were obtained from Jackson Laboratory and maintained on a CD1 outbred background. Unless otherwise specified, all experiments were performed using male and female mice on a CD1 outbred background, aged E12.5 to P30.
Wild animals	No wild animals were used in this study.
Reporting on sex	Both sexes were pooled for histological, scRNA-seq, and ultrasonic vocalization studies.
Field-collected samples	No field collected samples were used in this study
Ethics oversight	All animal procedures adhered to the guidelines of the University of Connecticut Health Center Animal Care Committee and the national and state policies.

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

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>