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Life Sciences Reporting Summary

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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.

   No statistical methods were used to pre-determine sample sizes but out sample sizes are similar to those reported in previous publications (Krishaswamy et al. 2015, Duan et al. 2014, Liu and Sanes 2017). At least 3 animals were analyzed per condition per genotype in each experiment and multiple independent measurements (cells) were taken from each animal. Exact numbers of animals and cells are provided in text and/or figure legends.

2. Data exclusions

   Describe any data exclusions.

   Exclusion criteria were pre-established for initial characterization of calcium responses in control animals (Supplementary Figure 6a-d) to eliminate noise due to technique. We counted only cells with quality index >0.45 and a z-score that was >1.0 for at least two consecutive time-points. For physiological comparisons across genotypes, all data were included. No other data were excluded.

3. Replication

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

   All data was replicable, with the number of replicates (cells and animals) provided in text and/or figure legends.

4. Randomization

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

   Samples were allocated by genotype.

5. Blinding

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

   No blinding was performed since phenotypes were assessed by definitive criteria that were applied consistently across all animals and cells.

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.

Generic functions on commercial or freely available software were used. They are as follows: ImageJ 1.49u, winDRP v1.6.4, Matlab R2015b, Imaris x64 7.4.0, Igor Pro 6.12A, R 3.1.3, Graphpad Prism 7.03.

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.

All unique materials are readily available from the authors or from standard commercial sources as stated in Methods.

9. Antibodies

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

All antibodies, supplier name, catalog number, working dilutions are reported in Methods. Antibodies used were as follows: chicken anti-GFP (1:1000, Abcam ab13970), rabbit anti-Tbr1 (1:1000, Abcam ab31940, McKenna et al. 2011), rabbit anti-Tbr2 (1:500, Abcam ab23345), goat FoxP2 (1:1000, Abcam), guinea pig FoxP1 (1:5000, Ben Novitch), goat Satb1 (1:1000, Santa Cruz Biotechnology sc-5989X), goat Pcsk2 (1:1000, R&D Systems AF6018, Supplementary Figure 4e) rabbit anti-mCherry (1:5000, Cai et al. 2012), mouse anti-Cre (1:500, Millipore MAB3120), 1:1000, goat anti-VAchT (1:1000, Millipore ABN100), mouse anti-Brn3a (1:500, Millipore Mab1585), guinea pig anti-VAchT (1:500, Promega G4481), guinea pig Rbpms (1:500, PhosphoSolutions 1832-RBPMS), goat anti-Brn3b (1:500, Santa Cruz Biotechnology sc-6026), mouse anti-Brn3c (1:250, Santa Cruz Biotechnology sc-81980), rabbit anti-Calbindin (1:10000, Swant CB38a), rabbit anti-CART (1:2000, Phoenix Pharmaceuticals H-003-62), Syt2 (1:250, ZIRC Znp-1), goat anti-Opn (1:500, R&D Systems AF-808), goat Sorcs3 (1:1000, R&D systems AF3067, Figure 5d), mouse PKCa (1:500, Abcam ab31), goat anti-Alcam (1:1000, R&D systems AF1172, Buhusi et al. 2009), goat anti-Neo1 (1:1000, R&D systems AF1079, Supplementary Figure 8c) and rabbit β-galactosidase (1:5000 Duan et al. 2014). Dylight405-, Alexa488-, Cy3- and Alexa647-conjugated secondary antibodies (1:1000) were obtained from Jackson Immunoresearch. Unless stated otherwise, these antibodies have been previously validated in Rousso et al. 2016.

10. Eukaryotic cell lines

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

No eukaryotic cell lines were used for results presented 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.

All mice used are reported in Methods. They are on a mixed CD1-C57BL6 background. No sex-specific differences were noted so animals were analyzed regardless of sex. Adult animals are defined as 21 days or older. Animals across developmental and adult stages were immunostained for expression analyses; all ages were reported in Results and Figure legends. Adults were used for calcium imaging. Electroporation and AAV injections were performed on postnatal day 0 pups; pups are sacrificed at P12-14. P5-6 mice were used for J-RGC RNAseq.

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 study did not involve human research participants.