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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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 (*n*) 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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 *d*, Pearson's *r*), 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	Nikon Elements (NIS ElementsAR ver. 4.6.0.) and Zeiss Zen software (2012 S4) were used to acquire images.
Data analysis	ImageJ2/FIJI, Photoshop ('screen') overlay method, or custom Python code were to contrast and overlay images as described in the Methods section. NUPACK 3.0.4 was used to calculate cross-hybridization probabilities of PER concatamers and Bowtie2: 2.2.4 Jellyfish 2.2.4, and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to further process sequences. Puncta were identified and assigned to single cells using CellProfiler 3.0 or custom MATLAB (version 2018a) pipelines (PD3D - all now available online). Cell segmentation was performed with previously published software (ACME: https://wiki.med.harvard.edu/SysBio/Megason/ACME), and plots were generated with Python (version 2.7) and Seaborn (version 0.8.1) or in R (version 3.5.1).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All raw and processed data will be made available upon request.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For experiments involving quantification of puncta, puncta intensity and numbers of signal positive cells in retina n=3 was chosen as the minimal replicate number, and sample size was determined by the number positive cells or puncta within the replicates. We determined this to be sufficient owing to internal control (specific staining of positionally defined cell types using known markers) and low observed variability between stained samples.
Data exclusions	Data were not excluded from analysis.
Replication	All replication attempts were successful and observed marker expression patterns were consistent with orthogonal methods and previously known results. For retinal probes used in this study, we find that in all cases the expression patterns were consistent with cell type-specific expectations from previous studies of RNA sequencing, as well as protein antibody stains. For final quantification, all samples were quantified for a minimum of three retinal sections with the exception of Figure 5D, where quantification of cell localizations are specific to the image shown.
Randomization	Retinas and sections used for imaging were selected randomly, however all cells and puncta that passed quality control were analyzed equally with no sub-sampling and thus, there was no requirement for randomization.
Blinding	Blinding was not possible as experimental conditions were evident from the image data. Quantifications were performed using computational pipeline applied equally to all conditions and replicates for a given probe. Thresholds for detecting puncta were chosen for each probe based on graphs with objective properties that appeared indistinguishable across conditions.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Anti-PKCa (MilliporeSigma P4334, lot #085H4848), used at 1:1500 Anti-Calretinin (MilliporeSigma AB1550, lot #2510177), used at 1:1000 Anti-GFP (Abcam AB13970), used at 1:750 Donkey anti-Chicken Alexa488 (Jackson ImmunoResearch Laboratories, 703-545-155), used at 1:500 from 0.625mg/mL stock solution Donkey anti-Goat Alexa647 (Jackson ImmunoResearch Laboratories, 705-605-147), used at 1:500 from 0.625mg/mL stock solution Donkey anti-Rabbit Alexa488 (Jackson ImmunoResearch Laboratories, 711-545-152), used at 1:500 from 0.625mg/mL stock solution
Validation	Anti-PKCa (MilliporeSigma P4334) antibody has been validated by MilliporeSigma by demonstrating immunoblotting on rat brain extract and inhibition of this signal with an immunizing peptide (see website). This antibody was also validated in our previous work (Shekhar and Lapan, 2016), where it was shown to specifically label rod bipolar cells based on overlap with rod bipolar cell-specific markers that were identified in Drop-seq data.

Anti-Calretinin (MilliporeSigma AB1550) has been validated for use in immunohistochemistry and western blotting, as stated on the MilliporeSigma product page.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MRC-5 (human, ATCC CCL-171) HEK293T cells (human, ATCC CRL-1573)
Authentication	None of the cell lines have been authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination but no indication of contamination was observed.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Wild-type CD1 mice (male and female) age P13, P17 or P25 were used for retina harvest.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	The mouse work was performed under the study protocol IS00001679, as approved by the Institutional Animal Care and Use Committee.

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