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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 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 (*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	No software was used to collect the data in this study.
Data analysis	FlowJo v10; Image J; GraphPad Prism v7.02; Partek Flow; Python (version 2.7); code for scRNAseq analysis is available at Github: https://github.com/Hongjie-Li/flyglia

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 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

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. Raw sequencing reads and preprocessed sequence data for Bulk RNAseq files have been deposited in GEO under accession code GSE168530 and scRNAseq reads and preprocessed sequence data have been deposited in GEO under accession code GSE168572. They both are available to the public.
Drosophila genome (version BDGP6) is available for download at https://aug2017.archive.ensembl.org/info/data/ftp/index.html
Drosophila genome (r6.10) is available for download at http://ftp.flybase.net/releases/.
scRNAseq datasets of Drosophila brain during aging published by Dr. Stein Aerts lab are available at https://scope.aertslab.org.

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	No statistical methods were used to calculate the sample size. Sample numbers are described in the corresponding figure legends. Sample sizes were determined according to our previous publications and literature in the field, as we are using similar experimental paradigms.
Data exclusions	No data exclusions were done in this study.
Replication	All the experiments were repeated at least twice, as specified in each figure legend.
Randomization	For Ecc15 treatments, flies with the same sex, age and genotype were randomly assigned to control or experimental groups. Where cohorts of animals with defined sex, age and genotypes were compared, animals were randomly collected from breeding cages, sorted according to sex and genotype, and combined into cohorts. As all animals in a cohort were analyzed, no randomization had to be performed in the data collection.
Blinding	To establish cohorts for experiments, the experimenter was not blinded to the genotype, age, or sex of the animals (genotyping and sexing was performed by visual markers, as is standard for Drosophila genetics). Image analysis, quantification, and data analysis were performed on samples that were blinded to the experimenter.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Primary antibodies and dilution used in this study: mouse anti-repo (DSHB 8D12, 1:100), mouse anti-NC82(DSHB, 1:50-1:100), rabbit anti-GFP(ClonTech, Cat# 632592, 1:500). Fluorescent secondary antibodies were bought from Jackson Immunoresearch (1:500). DAPI was used to stain DNA at 1:1000.
Validation	All the antibodies used in this paper are commercially available. Validation of these antibodies are available at the manufacturer's websites. The RRID and validation reference for each antibody is listed below: 1. mouse anti-repo (DSHB 8D12): AB_528448. https://dshb.biology.uiowa.edu/8D12-anti-Repo 2. Mouse anti-NC82 (DSHB): AB_2314866. https://dshb.biology.uiowa.edu/nc82 3. Rabbit anti-GFP (ClonTech, Cat# 632592): AB_2336883. https://www.labome.com/product/Takara-Bio-Clontech/632592.html

Animals and other organisms

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

Laboratory animals	For all the studies, we used adult females Drosophila melanogaster. Full genotypes and ages of flies used in each figure are listed in Supplemental Table 1.

Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	This study did not require ethics approval.

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For flow cytometry analysis in Extended Data Fig.2e, 20 brains were dissected for each biological replicate. Single cell suspension of each sample was prepared freshly, using the combination of papain and liberase (Li, et al. 2017. Cell). Cells was stained with eFluor™ 660 on ice (1:1000) on ice. After staining, cells were washed with 1x cold PBS followed by fixation, permeabilization and stainning with anti-GFP antibody (ClonTech, Cat# 632592, 1:500) following a previously published protocol (Cai, et al. 2018.), using eBioscience™ Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher Cat. No. 00–5523–00). DAPI was added to each sample at a final concentration 1µg/mL, to stain nuclei, and analyzed by BD Symphony flow cytometer. Fluorescent secondary antibodies were bought from Jackson Immunoresearch (1:500). For glia live sorting shown in Extended Data Fig.7g, about 100 brains were dissected for each replicate in cold Shields and Sang M3 insect medium (Sigma S8398) containing 10% fetal bovine serum (FBS; ThermoFisher,16000036). Brains were dissociated in the solution containing 300ul papain (Sigma, P4762; dissolved in 1xPBS to a final concentration of 100units/ml) and 4.1ul liberase TM solution (Roche, 5401119001; reconstituted with 1xPBS to a final centration of 2.5mg/ml) at 25°C, 1000rpm for 20min. Cells were stained with Calcein blue (ThermoFisher, c1429, 1:1000) for 20min on ice, washed, and resuspended in dissection buffer with SYTOX™ Deep Red Dead cell stain (ThermoFisher, S11381, 1:1000). GFP+ tdTomato+ cells and GFP- tdTomato+ cells were sorted into Trizol (ThermoFisher, 15596026) respectively with BD FACSAria™ Fusion.
Instrument	BD Symphony flow cytometer; BD FACSAria™ Fusion
Software	FlowJo v10 Software
Cell population abundance	The percentage of GFP+, mCherry+ population among total DAPI+ cells were quantified in Extended Data Fig.2e.
Gating strategy	Gating strategy for Extended Data Fig.2e is illustrated in the same panel with quantifications, as below: Forward versus side scatter (FSC vs SSC); side scatter height vs width (SSC-H vs SSC-W); forward scatter height versus width (FSC-H vs FSC-W); fixable viability dye (eFluor™ 660 to label dead cells before fixation) versus DAPI (labeling nuclei to exclude debris); mCherry versus GFP fluorescence channel (mCherry vs GFP). Gating strategy for Extended Data Fig.7g is illustrated in Supplementary Figure, and is listed as below: Forward versus side scatter (FSC vs SSC); forward scatter height versus width (FSC-H vs FSC-W); side scatter height versus width (SSC-H vs SSC-W); Calcein pacific blue (labelling live cells) versus Sytox APC (labelling dead cells); GFP versus YG-mCherry fluorescence channel.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.