nature portfolio

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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 (*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	NIS-Elements software (version AR 4.30.02), BD FACSDiva software (version 8.0.1), Zen blue edition (version 3.0)
Data analysis	Data analysis was performed using Python, version 2.7 with Python sklearn.decomposition.PCA module. Code available at https://github.com/Ruetz/Cas9_aging_NSC. Other analysis tools used include Prism (v8, Fiji (v2), EnrichR (https://maayanlab.cloud/Enrichr/), Flowjo (v8), QuPATH (version 0.2.3).

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 generated in this study have been deposited in NCBI GEO, with accession GSE189251 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE189251).

Figures 1, 2, 4 and Extended Data Figures 1, 2, and 4 of this study are based on the raw data which can be found under this accession number. Raw sequences for screen libraries are provided as FASTQ files and csv count files. For gene ontology searches with EnrichR, we searched the following databases: GO Biological Process 2018 (https://maayanlab.cloud/Enrichr/geneSetLibrary?mode=text&libraryName=GO_Biological_Process_2018), Molecular function 2018 (https://maayanlab.cloud/Enrichr/geneSetLibrary?mode=text&libraryName=GO_Molecular_Function_2018), Cellular components 2018 (https://maayanlab.cloud/Enrichr/geneSetLibrary?mode=text&libraryName=GO_Cellular_Component_2018). The gene essentiality databases can be found here: https://v3.ogee.info/#/home and http://cefg.uestc.cn/ceg. The single-cell dataset used for analysis can be accessed at: https://zenodo.org/doi/10.5281/zenodo.7338745.

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

Life sciences study design

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

Sample size	Sample size was decided based on previous experiments and prior literature using similar experimental paradigms as outlined in (Leeman et al. Science Vol 369, Issue 6381). We did not use power analyses and have clearly indicated this in Methods (Statistics section). We have clearly indicated cases where samples from independent experiments were combined, and have included all combined and non-combined data (and associated statistics) in Source Data.
Data exclusions	For the qNSC in vitro activation experiments, on rare occasions the cells did not activate at all (0% Ki67+, in both young and old conditions), and these samples were excluded from further analysis.
Replication	All data in this paper was replicated in at least one other independent experiment (see Source Data), with the exception of Figure 3c-d, Figure 4d, e and extended Figure 3d, 5b and 6o (one experiment performed in 4 animals for Figure 4d, e).
Randomization	For in vivo and in vitro screening experiments, mice/cells from each treatment group were randomized for which treatment they received.
Blinding	Blinding was generally not done for these figures. However, all of our quantifications were performed in an automated fashion using software tools (e.g. QuPATH image analysis, Python CasTLE analysis, Varioskan plate reader). We did not select areas to image. We imaged and quantified the entire olfactory bulb or SVZ region. We have indicated that no blinding was done for these figures in the Methods section.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Immunofluorescence: In vitro: GLUT4 (Abcam, 33780) 1:500, Ki67 (Invitrogen, 14-5698-082 clone SolA15) 1:500, STX4A (Santa Cruz Biotechnology, SC101301 clone QQ-17) 1:500. Alexa 488/594/647 conjugated antibodies, (Fisher Scientific, A21206, A21209, A31571) 1:500.
Immunofluorescence straining in brain sections in vivo: mCherry (Invitrogen, M11217 clone 16D7) 1:500, GFAP (Abcam, 53554) 1:500, GFP (Abcam, 13970) 1:500, GLUT4 (R&D Systems, MAB1262 clone 1F8) 1:500, Ki67 (Invitrogen, 14-5698-082 clone SolA15) 1:500, STX4A (Santa Cruz Biotechnology, sc-101301 clone QQ-17) 1:500, GFP (Abcam, 13970) 1:500, mouse IgG (Santa Cruz SC-3877, Lot: L1916) 1:500. Alexa 488/594/647 conjugated antibodies, (Fisher Scientific, A21202, A21206, A21209, A21447, A31571, A31573) 1:500, NeuN (Millipore, MAB377, clone A60) 1:500, S100a6 (Abcam, ab181975 clone EPR13084-69) 1:500, Tuj1 (Biolegend, 802001) 1:500, Olig2 (R&D Systems, AF2418) 1:100, Sox10 (Abcam, Ab180862 clone EPR4007-104) 1:100, Calretinin (Abcam, Ab244299) 1:500, Dcx (Cell Signalling Technology, 4604) 1:500.
Western blot: GLUT4 (Invitrogen, PA1-1065) 1:500. B-actin (Abcam ab6276) 1:40,000.
FACS: Ki67 (Ki67-APC (eBioscience, 17-5698-82) 1:300, GLUT4 (R&D Systems, MAB1262): diluted a 5:1 ratio with secondary anti-IgG AlexaFluor647, and the mix was added to live cells in culture at a dilution factor of 1:200.

Validation

The Ki67 antibody was validated by knockout in HeLa cells (by manufacturer) and in our manuscript by verifying that it only stained the nucleus of primary cultures of mouse activated NSCs (proliferative NSCs) and not quiescent NSCs (non-proliferative NSCs). The Dcx, Stx4a, S100a6, Olig2, Tuj1, Calretinin, and GLUT4 (Invitrogen, PA1-1065, used for western blot) antibodies were confirmed to stain the correct size band on western blot of rodent tissue/cells by the manufacturers. The mCherry/GFP antibodies were validated in mCherry-overexpressing human/mouse tissue and cell lines by western blot/immunofluorescence by the manufacturers. The NeuN, Tuj1, Olig2, Sox2, Calretinin, GLUT4(Abcam, 33780) and GLUT4(R&D Systems MAB1262, used for immunofluorescence and FACS) antibodies were validated by manufacturer by immunocytochemistry staining in rodent tissue, confirming correct morphological staining and regional specificity. The Ki67, Dcx and GFAP antibodies were also validated in vivo by examination of mouse coronal cryosections: Ki67 and DCX specifically marked cells in the SVZ neurogenic niche and along the rostral migratory stream (and no other regions), and the GFAP antibody displayed stereotypic cytoplasmic and projection GFAP staining present in both Ki67-positive (aNSCs) and Ki67- negative cells (qNSCs) but no Dcx+ cells (neuroblasts). Additionally, all three of these antibodies are widely cited in the literature. We also validated the GLUT4 antibodies that we used in immunocytochemistry (R&D Systems, MAB1262), FACS (R&D Systems, MAB1262) and western blot (Invitrogen, PA1-1065) by performing a CRISPR/Cas9-based knockout in this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	293T cell line from ATCC (CRL-3216)
Authentication	Not authenticated
Mycoplasma contamination	Tested negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study

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

Cas9-expressing mice (Cas9 mice) were obtained from Jax (https://www.jax.org/strain/024858). These mice (background C57BL/6N) constitutively express the Cas9 endonuclease and an EGFP reporter under the control of a CAG promoter knocked into the Rosa26 locus1. All screens in this study were performed with the Cas9 mice, including all NSC primary cultures and all in vivo work. We maintained a colony of Cas9 mice ranging in ages up to 28 months at the Stanford Comparative Medicine Building and the Neuroscience-ChemH building vivarium. As a negative control for the in vivo screens, male C57BL/6 mice obtained from the National Institute on Aging (NIA) Aged Rodent colony were used at 18-21 months old. NIA mice were habituated in the Stanford facility for at least 2 weeks prior to initiation of experiments. Mice were maintained under the care of the Veterinary Service Center at Stanford University under IACUC protocols 8661. Mice were housed in a 12 hour light/dark cycle, with ad libitum access to food/water, at 21o

	C and 50% humidity.
Wild animals	N/A
Reporting on sex	We have indicated the sex used in this study (both males and females were used).
Field-collected samples	N/A
Ethics oversight	At Stanford, all mice were housed in either the Comparative Medicine Pavilion or the Neuro Vivarium, and their care was monitored by the Veterinary Service Center at Stanford University under IACUC protocols 8661.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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 the genome-wide screen and for other qNSC reactivation experiments, we FACS-isolated proliferative cells (Ki67+) as follows. Cells were dissociated with Accutase (Stemcell Technologies, 07920) for 5 minutes, collected into conical tubes, and centrifuged at 300g for 5 minutes. Cells were resuspended in PBS at 5x10^7 cells in 1 mL (or 1x10^5 cell in 100 µL), and then 9 mL (or 900 µL) ice-cold 100% methanol was added and cells were agitated for 15 minutes at 4°C. Cells were then centrifuged at 500g for 5 minutes and resuspended for a wash in 3 mL PBS and centrifuged again at 500g for 5 minutes. Cells were then resuspended in 3.5 mL staining solution: Ki67-APC (eBioscience, 17-5698-82) 1:300 in PBS, 2% fetal bovine serum (FBS) (Gibco, 10099141) at 4°C. Samples were agitated for 30 minutes at room temperature in the dark, and then 10 mL PBS was added prior to centrifugation at 700g for 5 minutes. Samples were then resuspended (25 mL per 5x107 cells) in FACS buffer: PBS, 2% FBS, DAPI (Fisher Scientific, 62248, 1 mg/mL) 1:5000. Each sample was filtered with FACS-strainer cap tubes (Fisher, 08-771-23), just prior to FACS sorting. Cells were sorted on an Aria BD FACS Aria with a 100 µm nozzle at 13 psi and Flowjo (v10) software was used for data analysis.
Instrument	All cell sorting was performed using BD Aria II or BD PICI machine models housed in the Stanford Shared FACS Facility.
Software	Flowjo (v8) software was used for data analysis.
Cell population abundance	The %Ki67+ cells ranged from 2-40% depending on treatment and experimental setup.
Gating strategy	Gating was determined using fluorescent-minus-one controls for each color used in each FACS experiment to ensure that positive populations were solely associated with the antibody for that specific marker.

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