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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	cellSens Dimension version 1.18 (Olympus), Zen 2.6 Blue edition (Carl Zeiss Microscopy)
Data analysis	Statistical analyses were performed using GraphPad Prism (Graphpad Software v. 6 .0 and 8.0)

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 upon request. 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 data generated or analysed during this study are included in this article and its Supplementary Information files, or are available from the authors upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical method was performed to predetermine sample size. Sample sizes were determined based on reproducibility between biological replicates and individual experiments and on magnitude and consistency of measurable differences between groups.
Data exclusions	No data were intentionally excluded from the analyses. Pr-established criteria for determining outliers were based on technical reasons and verified with the Grubb's outlier test for exclusion from analysis.
Replication	All data are the result of independently-repeated experiments with independent biological sample. Experiments were repeated independently a minimum of three times with similar results in repeated experiments. All attempts at replication were successful assuming the technical reliability was acceptable within the experiment. Data reported in the manuscript are from biological replicates.
Randomization	Randomization of samples was not relevant to the studies performed. Samples were allocated based on indicated treatment groups for analyses such as western blotting or luciferase activity.
Blinding	Blinding was not possible during most of data collection as investigations required information on the nature of groups or treatments. Blinding was used during scoring of competitive Bimolecular Fluorescence Complementation (BiFC) assays as stated in the Methods.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials are readily available from authors

Antibodies

Antibodies used	Rabbit monoclonal anti-RCAS1, Cell Signaling, 12290, Lot 3 ,1:100 Rabbit polyclonal anti-PDI, Cell Signaling, 3501, Lot 3,1:100 Goat anti-Rabbit Alexa Fluor 647, ThermoFisher, A-21244, 1:1000 DyLight 649-conjugated goat anti-rabbit, Jackson ImmunoResearch,111 995-003, 1:1000 Mouse monoclonal anti-ABHD5, Abnova, H00051099-M01, Lot H5091-1F3, 1:1000 Goat polyclonal anti-PNPLA3, Everest, EB08402,Lot G2R1, 1:10000 Rabbit polyclonal anti-PNPLA3, Abcam, ab81874, Lot GR3209727-2, 1:1000 Rabbit polyclonal anti-Gaussia luciferase, Nanolight, 401P, Lot 1107, 1:5000 Rabbit polyclonal anti- alpha/beta tubulin, Cell Signaling, 2148S, Lot 7, 1:1000 Mouse anti-Goat IgG HRP linked Light Chain Specific, Jackson ImmunoResearch, 205-032-176, Lot 120312, 1:10000 Donkey anti-Mouse IgG HRP linked, Jackson ImmunoResearch, 715-035-150, 1:10000 Goat anti Rabbit IgG HRP linked, Cell Signaling, 7470, Lot 26, 1:10000 Rabbit IgG, Vector, I-1000, Lot X0720

Rabbit polyclonal anti-ABHD5, Proteintech custom antibody made against full length mouse ABHD5

Validation

Antibody validation for Western blot analyses involved confirmation that the band corresponded to the reported molecular mass following gel migration.
Antibodies against ABHD5 were initially verified using a cell line with shRNA knockdown against ABHD5. Antibodies against PNPLA3 were verified using brown adipose tissue lysate as a positive control and cell lines that lacked known expression of PNPLA3.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	COS7, 293A and AML12 cells were from ATCC. Immortalized brown adipocytes were a gift from Bruce Speigelman (Harvard).
Authentication	Cell lines were derived from authenticated stock from ATCC. No additional verification was performed.
Mycoplasma contamination	Cell lines were previously determined to be free from Mycoplasma contamination. Generation of novel stable cell lines were cultured in antibiotics against Mycoplamsa (10 ug/ml Ciprofloxacin) prior to establishing in the lab.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells were used.

Animals and other organisms

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

Laboratory animals	Males C57BL/6J mice 8-12 weeks of age were used.
Wild animals	The study did not involve the use of wild animals.
Field-collected samples	The study did not involve field-collects samples.