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	immunofluorescence images were acquired using Zeiss Axio Scan 7. Protein bands for Western blotting were visualized with the MBI Fusion FX7 system.
Data analysis	For Two-sample Mendelian Randomization analysis, we used TwoSampleMR R package (v0.5.6) from https://mrcieu.github.io/twosampleMR/. For RNA-Seq analyses, we used MultiQC (v1.12), trim galore (v0.6.5-1), Salmon (v1.10.1), DESeq2 (v1.38.3), GOstats (v2.64.0), KEGG.db (v3.2.3), cnetplots for R. RNA Sequencing visualizations were performed in R. The single cell RNA-Seq data used for the analyses described in this article were obtained from the NCBI Sequence Read Archive under reference number SRP14547526. The Cell Ranger was used to perform sample qualifying, alignment, filtering, counting, and aggregation using the Linux system or the R and RStudio software. Clustering and gene expression were visualized with 10X Genomics Loupe Browser (v.6.5.0). Statistical analyses were performed in Prism (version 10), R (Version 4.2.3), or RStudio software (Version 1.3.1056). MATLAB (v2022b) were used for analyses of open-field tests. Western Blot images were quantified using Image J software

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

FASTQ files from these scRNA-seq libraries areavailable at the Pique-Regi labwebpage(http://piquelab.grid.wayne.edu/), as well as the Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra/) (SRA: SRP145475). Scripts for data processing are available through GitHub (https://github.com/RBBurl1227).
The data that support the findings of this study are available from the corresponding author upon request

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	Unless otherwise states only male mice were used in this study. For mouse experiments where male and female mice were compared, age-matched litter mates were used. Biological sex was considered, and reported, where possible in human analyses.
Reporting on race, ethnicity, or other socially relevant groupings	Not Applicable
Population characteristics	Children with obesity who were enrolled in the Canadian Pediatric Weight management Registry (CANPWR) at the McMaster site were included in this study. Study participants with an available fasting blood sample at their initial visit and a clinical diagnosis of anxiety but without use of antipsychotic or antidepressant medications (n=23) were compared to those without any diagnosis of anxiety and also free from use of medications (n=24). Participants were matched for age (12.50+/-2.97 and 12.44+/-2.86 years of age), sex (12/11 and 12/12 male/female), body weight (87+/-31.88 and 88+/-35.32 kg), and body mass index (33.18+/-6.41 and 34.08+/-8.07) between control and participants with anxiety, respectively.
Recruitment	Not Applicable
Ethics oversight	Animal studies were carried out at McMaster University (210104), University of Toronto (21–467 and 24-0362H), or Washington University in St. Louis (20-0139). All animals used in the study were housed and cared for in accordance with the Guidelines for Animal Use at McMaster University and were approved by the McMaster University Animal Ethics Research Board or their respective facilities Ethics Boards.

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	No statistical test was used to determine sample size but sample size for all animal studies were based on well-established protocols previously performed in authors' labs (Wang et a., Nature, 2023; Day et al. Nature Metabolism, 2019; McCall et al. 2015, Nueon; McCall et al. 2017, eLife; Blondin et al. 2022, Cell Metabolism) and provide adequate power to detect the substantial effect while ensuring no more animals that necessary were used. In vitro sample size were based on previous experience (Townsend et al. 2021, FASEB; Day et al. 2019, Nature Metabolism). Sample size was also informed by animal availability, homogeneity, and consistency of characteristics of selected animals.
Data exclusions	Data were excluded only if statistically confirmed as outlier: 1 sample from BAT in Figure 1G; 2 mice Figure 1H; 1 mouse from Figure 3B, 1 mouse from Extended Data Figure 1A. One participant was excluded from pediatric analysis as it was inappropriately included by human error. Exclusions did not affect interpretation of results.
Replication	Nearly all experiments were completed across at least 2 independent cohorts. All cell-based experiments are from 3 independent experiments. All attempts at replication were successful.

Randomization	Prior to experimentation animals were randomly assigned to groups. For human participants the recruitment, collection, and sample selection was completed by a blinded researcher. Participants were matched by researcher prior to analysis.
Blinding	Investigators were blinded to experimental groups during tissue collection. During behavioural tests blinding was not possible during experiment as drug administration was required by subsequent behavioural analyses of tests was performed by blinded researcher.

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	Phosphorylated PKA substrates (1:1000 dilution, Cell Signalling Technology, Catalogue #9624, lot 10) beta-tubulin (1:1000 dilution, Abcam, catalogue number #ab4074) c-Fos (1:1000 dilution, Cell Signaling Technology Cat# 2250s) TH (1:1000dilution, Aves Lab, Cat#TYH)
Validation	All antibodies were used in accordance to the manufacturer guidelines. Western blotting remains one of the most common scientific methods for monitoring protein expression in cells or tissue. The accuracy of western blot results relies heavily of the quality of the primary antibody employed in the immunoblotting. Cell Signaling Technology (CST) provides the highest quality primary and secondary antibodies available for western blotting. CST™ antibodies are produced in-house and validated extensively according to a rigorous protocol. Validation Steps Include Examination of several cell lines and/or tissues of known expression levels allows accurate determination of species cross-reactivity and verifies specificity. Treatment of cell lines with growth factors, chemical activators or inhibitors, which induce or inhibit target expression, verifies specificity. Phosphatase treatment confirms phospho-specificity. The use of siRNA transfection or knockout cell lines verifies target specificity. Side-by-side comparison of lots to ensures lot-to-lot consistency. Optimal dilutions and buffers are predetermined, positive and negative cell extracts are specified, and detailed protocols are already optimized, saving valuable time and reagents.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Bone marrow-derived macrophages were isolated from mice and confirmed by gene expression signatures, as done previously in our lab (Day et al. 2023, Cell Reports; Morrow et al. 2022, Cell Metabolism) White adipocytes were generated and differentiated as previously described by our lab (Mottillo et al. 2016, Cell Metabolism 10.1016/j.cmet.2016.06.006)
Authentication	Cell lines were authenticated by appropriate gene signatures and physiological responses herein.
Mycoplasma contamination	Not Applicable
Commonly misidentified lines (See ICLAC register)	Not Applicable

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	Germline GFRAL knockout mice (Gfral-/-), b1, b2, b3-adrenergic receptors triple knockout mice, Atgl/Pnpla2-floxed and Atgl/Pnpla2 knockout mice used in this study were on a C57bl/6j background, all males unless otherwise stated, and between the ages of 16-24 weeks for experiments. Mice were housed in temperature controlled either ambient (~22C at 40-60% relative humidity) or thermoneutral conditions (~29C).
Wild animals	No wild animals were used.
Reporting on sex	Unless otherwise stated only male mice were used in this study.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Guidelines for Animal Use at McMaster University and were approved by the McMaster University Animal Ethics Research Board (AUP 210104) or their respective facilities Ethics Boards

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Clinicaltrials.gov identifier NCT02811289.
Study protocol	Full protocols for experiments have been previously published. Pediatric samples : Morrison et al. 2014, BMC Pediatrics, 10.1186/1471-2431-14-161
Data collection	Please see previous publications for detailed descriptions of data collection and outcomes.
Outcomes	Please see previous publications for detailed descriptions of data collection and outcomes.

Plants

Seed stocks	Not Applicable
Novel plant genotypes	Not Applicable
Authentication	Not Applicable