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

| Microarray data: Mouse Gene 2.0 ST arrays, GeneChip Scanner 3000 7G (Thermofisher Scientific) |
| Western blots: Odyssey Infrared Imaging System (LI-COR) |
| Fluorescent microscopy: Leica TCS SP5, AxioScan.Z1 digital slide scanner |
| Indirect calorimetry: PhenoMaster indirect calorimetry system (TSE systems) |
| Clinical chemistry: Beckman-Coulter AU480 autoanalyzer (Beckman-Coulter) |
| Magnetic resonance imaging: preclinical MRI BioSpec 94/20 USR (Bruker) |
| Micro computed tomography: Micro-CT system Bruker Skyscan 1172 (Bruker) |

Data analysis

| Transcriptome analysis: Transcriptome Analysis Console (4.0.1.36, Thermofisher Scientific), R software including limma package |
| Western blot: Image studio lite v5.2 |
| Immunohistochemistry: Image J, Definiens Developer XD2 |
| Magnetic resonance imaging: ParaVision 6.01, ImageJ |
| Micro computed tomography: 3D Dataviewer, 3D Slicer |
| Statistical analysis and graphs: Graphpad prism v8.1.2 |

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

Raw transcriptomics dataset described in the current study data can be found in the GEO database with accession number GSE128504.

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 sizes for each experimental group are provided in the methods section and the figure legends. |
|----------------------|--------------------------------------------------------------------------------------------------|
| Data exclusions      | No data was excluded.                                                                            |
| Replication          | All data available in the present study were from biological replicates and any attempt at replication was successful. |
| Randomization        | Randomization was not possible. All samples were grouped based on genotype.                       |
| Blinding             | The lack of eyes in homozygous mutant mice was a stark observable feature due to which blinding of the genotype was not possible. |

Behavioural & social sciences study design

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

| Study description | Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study). |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Research sample   | State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source. |
| Sampling strategy | Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed. |
| Data collection    | Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection. |
| Timing             | Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort. |
| Data exclusions    | If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established. |
| Non-participation  | State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation. |
| Randomization      | If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled. |
Ecological, evolutionary & environmental sciences study design

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

**Study description**
Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

**Research sample**
Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

**Sampling strategy**
Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.

**Data collection**
Describe the data collection procedure, including who recorded the data and how.

**Timing and spatial scale**
Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken.

**Data exclusions**
If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

**Reproducibility**
Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.

**Randomization**
Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.

**Blinding**
Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

**Did the study involve field work?**
- Yes
- No

**Field work, collection and transport**

**Field conditions**
Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

**Location**
State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

**Access and import/export**
Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

**Disturbance**
Describe any disturbance caused by the study and how it was minimized.

**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 | Relevant information on all antibodies used in the present study are described in supplementary table 3. |
|-----------------|-----------------------------------------------------------------------------------------------------|
| Validation      | Validation of the antibodies was provided by the manufacturer.                                      |

## Animals and other organisms

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

| Laboratory animals | Mice on the C3HeB/FeJ background strain, carry the wild-type Pax6 or mutated Pax6 (Leca2 mutation) are described in the methods section. |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Wild animals       | No wild animals were used in the study.                                                                                                                                                        |
| Field-collected samples | The study did not involve samples collected from the field.                                                                                                                                    |
| Ethics oversight   | All experimental procedures were performed in accordance with German and European Union guidelines.                                                                                        |

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