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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	No software used for data collection.
Data analysis	All softwares we used in this study have been described in Methods.

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 sequence data from the fall webworm genome project have been deposited in GenBank under an accession code PKRV00000000. Transcriptome data has been deposited in SRA under SRR6433091. Genomic sequencing data of other lepidopteran species has been deposited in SRA under SRR6432897, SRR6432898, and SRR6432899.

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	For de novo sequencing, all libraries with short-inserts were derived from ONE single individual to limit the heterozygous level which may cause problems to assembling. For RNA sequencing, we pooled a varying quantity of samples, from 3 to ~300, to represent each developmental stage or tissue, respectively, depending on the actual size of tissues at that stage; e.g., some tissues such as the silk glands at the 1st instar larvae or fat bodies are quite small, thus hundreds of individuals were dissected to collect enough RNA; we sampled from at least three individuals, with independent preparation of RNA, for some large tissues such as the silk glands at the last instar larvae or the whole body of pupae. For pilot resequencing, we sampled 20 individuals at five distinct locations (2 males and 2 females for each location). For CRISPR/Cas9 experiments, we injected at least 400 eggs for genetic manipulation of each gene. The injected quantity was based on our preliminary experiences on the actual hatching ratio and mutation ratio of injection with fall webworms.
Data exclusions	No data was excluded.
Replication	For the CRISPR/Cas9 experiments, we observed mutagenesis-induced phenotypes for each gene from independently injected individuals and only reported phenotypes that were presented by multiple individuals for each gene. For profiling silkglands between webworms and silkworms, we sequenced three biological replicates of transcriptome samples for each time point.
Randomization	For phenotypic comparisons between the fall webworm and the silkworm, laboratory-maintained wildtype lines of the fall webworm and the silkworm were reared under a standard condition independently. Individuals of abnormal phenotypes or developmental sizes were eliminated from the colony. At the corresponding stage, individuals were randomly selected from the colony for dissection and imaging, without any further filtering steps.
Blinding	Not applicable in this study.

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

Animals and other organisms

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

Laboratory animals	Not applicable in this study.
Wild animals	Related information has been listed in Supplementary Tables 9 and 15.
Field-collected samples	Not applicable in this study.