

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

Ubuntu 18.04
BLASTP 2.11.0+
Perl 5.26.2
Python 3.6.5
R 4.0.3
R studio 1.3.01093

Data analysis

Canu v2.2
Racon v1.5.0
Pilon v1.24
Hic-pro v2.10
3D-DNA v180419
Juicer v1.6.2
Juicebox v1.9
RepeatModeler v2
RepeatMasker v4.05
AUGUSTUS v3.2.3
MAKER2
MaxQuant v1.3.0.5

BSMAP 2.90
SAMtools v0.1.19
MACS2
Seurat v4.0.6
Monocle v2.22.0

More information and details were in the “Materials and methods” and “Supplementary Note”.

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](#)

All high-throughput sequencing raw data in this project were deposited into the CNGB Nucleotide Sequence Archive (CNSA) of the China National GeneBank DataBase (CNGBdb, <https://db.cngb.org>) and are available through BioProject ID CNP0002864 (<https://db.cngb.org/search/project/CNP0002864/>). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the iProX partner repository100 with the dataset identifier PXD038734. The single-cell and spatial metrices have been deposited in FigShare (https://figshare.com/articles/dataset/Single_cell_matrices_zip/20399475, https://figshare.com/articles/dataset/Spatial_Transcriptomics_zip/20399580). The genome assembly, gene annotation, and multiomics analysis results of *Trichonephila clavata* are also available on SpiderDB (<https://spider.bioinfotoolkits.net>).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

mm area, so we used this slice. For the ease of readers' understanding, we hand-painted the defective parts of this tissue section of interest without altering the authenticity of the data (Supplementary Fig. 20d).

Randomization

All samples were randomly allocated to experimental groups.

Blinding

Blinding does not apply to this study. Blinding is not necessary because this study focuses on the multiomics of spider Malpighian tubule.

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

Methods

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

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

A nondiapausing silkworm (*Bombyx mori*) strain, D9 L, at the stage of the fifth instar and pupa was used in this study.

Wild animals

The adult golden orb-weaving spiders, *Trichonephila clavata* (basonym: *Nephila clavata*, Nephilidae, Trichonephila), were captured from the wild in Dali City, Yunnan Province, China. Spider samples were captured from web directly by using bamboo stick. Tissues of all samples were dissected in PBS buffered saline and stored in 1.5mL microtubes at -80 °C until required.

Reporting on sex

All findings are applicable to female spiders, because a female orb-weaving spider can have up to seven morphologically differentiated types of silk glands.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

No ethical approval was obtained as it is not required for working on an invertebrate in China.

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