nature portfolio

Corresponding author(s): Hai-Jun Xu

Last updated by author(s): Jun 13, 2026

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	Single-cell RNA sequencing data were collected using the 10x Genomics Chromium platform. Sequencing was performed on an Illumina NovaSeq 6000 system. Confocal images were acquired using a Zeiss LSM 810 microscope with Zeiss Zen software. Images were captured using a Leica DVM6 microscope with Leica Application Suite X (LAS X) software. Flow cytometry data were acquired using a BD FACSDiscover S8 instrument with BD CellView (v.1.3.6).
Data analysis	Raw scRNA-seq reads were processed, aligned, and quantified using CellRanger (v6.1.0). Downstream single-cell analysis, including normalization, integration, and clustering, was performed using Seurat (v4.3.0) in R (v4.2.1). Batch effect mitigation was handled using Harmony and Seurat's CCA-based workflow. Doublet detection was performed with DoubletFinder (v2.0.3). Gene regulatory networks were inferred using pySCENIC (v0.12.1). For bulk RNA-seq, reads were aligned using STAR and quantified with RSEM, followed by differential expression analysis using DESeq2 (v1.20.0). Flow cytometry data were modeled using FlowJo (v10.10.0) with the Watson pragmatic algorithm. Phylogenetic analysis utilized OrthoFinder (v2.5.5), MAFFT (v7.520), trimAl (v1.4.1), and IQ-TREE (v2.2.3). Statistical analyses and plotting were conducted using GraphPad Prism (v8.0.2) and ggplot2. The R analysis code used for data processing, analysis and visualization is available on GitHub [https://github.com/wanyizju/Pyap-wing-singlecell-2026] and has been archived in Zenodo under DOI: https://doi.org/10.5281/zenodo.19603850. A detailed README.md file provides execution instructions.

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 data are available in the manuscript or the supplementary materials. All sequencing reads for the bulk RNA-seq and scRNA-seq have been deposited into the NCBI Sequence Read Archive (SRA) under the BioProject accession PRJNA1477850 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1477850], and are also available in the Genome Sequence Archive79 at the National Genomics Data Center80 China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA028437) [https://ngdc.cncb.ac.cn/gsa/browse/CRA028437]. The reference genome sequences were obtained from GenBank under the following accession numbers: GCA_039877355.1 for the firebug P. apterus and GCA_014356525.1 for the brown planthopper N. lugens. The reference genomes of both species, functional annotations of protein-coding genes, and the processed gene expression matrices have been deposited in Figshare [https://doi.org/10.6084/m9.figshare.30091297.v1]. Source data are provided with this paper.

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	The study did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	The study did not involve human participants.
Population characteristics	The study did not involve human participants.
Recruitment	Not applicable.
Ethics oversight	The study used only insects (Pyrrhocoris apterus and Nilaparvata lugens), which do not require ethical approval for research in China. All experiments were conducted following relevant institutional and national guidelines.

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	For scRNA-seq, three biological replicates were used per sample to ensure robust capture of cellular heterogeneity. For flow cytometry, six independent biological replicates were performed to provide sufficient statistical power. All sample sizes (n) are explicitly stated in the figure legends and the Methods section.
Data exclusions	No data were excluded from the analysis. All quality control metrics for scRNA-seq were pre-established, and only cells meeting these criteria (e.g., gene counts, UMI counts, and mitochondrial gene percentage) were retained for downstream analysis.
Replication	For scRNA-seq and bulk RNA-seq, three independent biological replicates were used for each group. Flow cytometry was performed with six independent biological replicates per group. Forewing cell-number quantification was performed with three independent biological replicates. EdU staining was repeated three times independently replicates. Sample sizes for RNAi phenotypic assays are provided in the figure legends and Source Data file.
Randomization	Insect samples of the same developmental stage were randomly allocated into experimental and control groups (e.g., for RNAi and pharmacological treatments). Given the synchronized rearing conditions and high genetic uniformity of the laboratory colonies, additional randomization was not deemed necessary.
Blinding	Blinding was not relevant to this study as experimental groups (e.g., wild-type vs. CRISPR/Cas9 mutants, or Gfp-RNAi vs. target gene-RNAi) were inherently defined by distinct genotypes or experimental treatments required for execution.

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	Anti-Digoxigenin-AP (Cat# 11093274910, LOT#32871924, Roche) was used at 1:2000 dilution or the detection of DIG-labeled RNA probes in whole-mount in situ hybridization.
Validation	The anti-DIG-AP antibody is widely used and validated by the manufacturer for the detection of digoxigenin-labeled nucleic acids in applications such as in situ hybridization. Please refer to the manufacturer website [https://www.sigmaaldrich.cn/CN/en/product/roche/11093274910].

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	Firebug (Pyrrhocoris apterus): Fifth-instar nymphs (both wild-type and PaInR2 mutants) were used for scRNA-seq and functional experiments. Brown planthopper (Nilaparvata lugens): Fifth-instar nymphs (both wild-type and NlInR2 mutants) were used for scRNA-seq and functional experiments.
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was not considered in the study design. Fifth-instar nymphs of both sexes were used for scRNA-seq and phenotypic analysis.
Field-collected samples	The P. apterus population was originally collected from Urumqi, China, in 2020. The N. lugens population was originally collected from Hangzhou, China, in 2008 and maintained in the laboratory for 17 years.
Ethics oversight	No ethical approval or guidance was required. This study utilized only insects (Pyrrhocoris apterus and Nilaparvata lugens), which are not subject to ethical committee oversight for animal research under current national and institutional guidelines in China.

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

Plants

Seed stocks	Red clover seeds (Trifolium pratense L.) and rice seedlings (variety: Xiushui 11) were used solely as food sources for rearing the insects (P. apterus and N. lugens, respectively). These materials were obtained from commercial sources.
Novel plant genotypes	Not applicable. No novel plant genotypes were produced or used in this study.
Authentication	Not applicable.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Wing buds were dissected from fifth-instar wild-type and PaInR2De4 firebug nymphs. Cells were suspended in cold Sf-900 II SFM medium, centrifuged at 500g for 5 min, and fixed in an ethanol:PBS (7:3, v/v) mixture for 2 hours on ice. Prior to analysis, cells were permeabilized with 0.1% Triton X-100 and stained with DAPI at for 15 min.
Instrument	Data collection was performed using a BD FACSDiscover S8 instrument.
Software	Data were acquired using BD CellView (v.1.3.6) and analyzed using FlowJo (v.10.10.0).
Cell population abundance	Approximately 10,000 singlet events were acquired per sample to ensure accurate estimation of cell cycle fractions. The purity of the singlet population was ensured by stringent pulse-geometry gating.
Gating strategy	Target cells were first isolated from debris using side scatter (SSC-H) vs. light-loss (LightLoss-H) gating. Doublets and multiplets were subsequently excluded using pulse-geometry gating on the DNA channel (UV5(460)-W vs. -A and -W vs. -H) to ensure only single cells were analyzed.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.