

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All gene sequences used to perform HCR and CRISPR-Cas9 were collected from NCBI. All the gene IDs and the probes used are provided in the supplementary file.
Data analysis	Raw reads were analyzed using fastqc and cleaned using bbdud (Bushnell, 2014)with the parameters ktrim= r, k = 23, mink = 11, hdist = 1, tpe, tbo. Cleaned reads were aligned using B. anynana ncbi reference genome assembly (txid: 110368) using Hisat2 (Kim et al., 2019) and mapped to reference B. anynana gtf file obtained from ncbi (txid: 110368) using stringtie (Pertea et al., 2015). A gene count matrix table was created from the stringtie files using pydr.py (Pertea et al., 2015) package using python2.7 followed by analysis using DESeq2 in R (Love et al., 2014). Volcano plots were created using EnhancedVolcano package in R.

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](#)

The sequencing data generated in the paper have been submitted to NCBI under the bio-project PRJNA948390.

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

NA

Reporting on race, ethnicity, or other socially relevant groupings

NA

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

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](https://www.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

Three sets of laser microdissection tissue samples were used for the RNASeq and DESeq analysis.

Data exclusions

NA

Replication

HCR and CRISPR-Cas9 experiments were repeated for each gene at least three times to ensure replicability.

Randomization

NA

Blinding

NA

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

Methods

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

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

Antibodies

Antibodies used	Optix (1:3000, rat), and Spalt (1:20000, guinea pig GP66.1)
Validation	Both the antibodies used in the study have been verified multiple times in multiple studies in different butterfly species. 1)Martin, A., McCulloch, K. J., Patel, N. H., Briscoe, A. D., Gilbert, L. E. and Reed, R. D. (2014). Multiple recent co-options of Optix associated with novel traits in adaptive butterfly wing radiations. <i>Evodevo</i> 5, 1–13. 2)Oliver, J. C., Tong, X. L., Gall, L. F., Piel, W. H. and Monteiro, A. (2012). A Single Origin for Nymphalid Butterfly Eyespots Followed by Widespread Loss of Associated Gene Expression. <i>PLoS Genet.</i> 8,. 3)Banerjee, T. Das and Monteiro, A. (2020). Molecular mechanisms underlying simplification of venation patterns in holometabolous insects. <i>Development</i>.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA