

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	Genomic DNA from Brassica napus accessions was sequenced using Oxford Nanopore Technologies (ONT) ultra-long-read sequencing, PacBio long-read sequencing and Illumina short-read sequencing. RNA-seq and population resequencing datasets used in secondary analyses were obtained from previously published datasets as cited in the manuscript. No custom software was used for instrument-level data collection.
Data analysis	Genome assembly and annotation analyses used Hifiasm v0.25, RagTag v2.1, NextDenovo v2.5.2, TGS-GapCloser v1.2.1, NextPolish v1.4.1, tidk, quarTeT v1.2.5, EDTA v2.2.2, HISAT2 v2.2.1, StringTie v2.2.1, Trinity v2.1.1, PASA v2.5.3, TransDecoder v5.7.1, IsoQuant v3.3.1, GeMoMa v1.9, BRAKER3, EvidenceModeler v2.1.0, SynGAP v1.2.5 and TESorter v1.4.7. Assembly and annotation quality was evaluated using BUSCO v5.5.0, OMArk, Salmon v1.4.0, KAT v2.4.2, minimap2 v2.27, Merqury v1.3, BlobTools v1.1 and Juicer v1.6.2. Comparative and population genomic analyses used MUMmer v4.0.0beta2, BEDtools v2.27, Circos v0.69-8, NLR-Annotator v2.0, DIAMOND v2.2.5, OrthoFinder v2.3.8, MUSCLE v3.8.31, Gblocks v0.91b, RAXML v8.2.12, WGDI, PAL2NAL, PAML, Minigraph-Cactus, SyRI, vg toolkit, SeqKit v2.8.0, CD-HIT-EST, RepeatMasker and BLAST+ v2.9.0. Association analyses used Python statsmodels, BWA v0.7.17, SAMtools v1.9, PLINK v1.9, LD-KNN and GEMMA. Custom genome assembly and annotation scripts are available at the GitHub (https://github.com/Wanjie-Feng/GenomeAssembly_Scripts). The version of the scripts used in this study (v1.0.0) has been permanently archived in Zenodo and is available at https://doi.org/10.5281/zenodo.22846774 .

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 raw sequencing data, genome assemblies and annotation files generated in this study have been deposited in the NCBI Sequence Read Archive under BioProject accession PRJNA1422850. The dataset includes ONT ultra-long reads, PacBio reads, Illumina short reads used for polishing, chromosome-level genome assemblies and corresponding annotation files. All assembled genomes, gene annotations and downstream analysis results are also available through the BnaScope database (<https://rgmi.hzau.edu.cn/bnascope/>) for browsing and bulk download. Custom scripts used for genome assembly and annotation are available at https://github.com/Wanjie-Feng/GenomeAssembly_Scripts.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No statistical method was used to predetermine sample size. Sample sizes were determined by the availability of genomic resources, population datasets and experimental materials appropriate for each analysis. The pan-genome analysis included 22 <i>B. napus</i> genome assemblies, including eight assemblies generated in this study and 14 published genomes. Population-level SV and association analyses used 505 <i>B. napus</i> accessions. Logistic-regression analyses of homoeologous gene expression were performed across 88 tissue/developmental-stage comparisons. Sample sizes for individual experimental validations are reported in the corresponding figure legends or Methods.
Data exclusions	Sequence and variant filtering criteria were defined as part of the analysis pipelines.
Replication	Genome assembly and structural-variant results were evaluated using multiple independent approaches. Seven randomly selected large insertion/deletion SVs were experimentally validated by PCR across 14 <i>B. napus</i> accessions, yielding a genotype prediction accuracy of 91.8%. Selected SVs at known loci were additionally validated using long-read evidence. Functional validation of BnaPYRD included two independently generated CRISPR/Cas9-edited lines and three overexpression lines.
Randomization	Random allocation was not applicable, as the study focused on naturally occurring genetic variation among rapeseed accessions. Samples were grouped based on genetic background and population structure rather than experimental treatment.
Blinding	This study did not involve experimental group assignment; all analyses were based on genotype and phenotype. Therefore, blinding was not applicable.

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	Involvement 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	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	All B. napus accessions were maintained by the National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University.
Novel plant genotypes	No novel plant genotypes were generated in this study.
Authentication	N/A