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Last updated by author(s): Jun 26, 2024

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	No software used for data collection.
Data analysis	Multiple published software packages were used in the analysis including: Assemblytics v1.2.1, Augustus v3.3.3, BBMap v37.93 & v37.28, BCFtools v1.9, bedtools v2.29.2, BFC v181, BLASTP v2.2.26 & 2.3.0+, BUSCO v3.0.2, BUSCO v5.2.2, Cuffcompare v2.2.1, cutadapt v3.3, EVidenceModeller v1.1.1, Fastuniq v1.1, findGSE v1.94, GeneMark v4.35, GenomeThreader v1.7.1, GEMMA v0.98.5, GMAP v2018-07-04, hifiasm v0.11-r302 & v0.15.5-r350, lastz (v1.04.03, MAFFT v7.490, , Merqury v1.3,Minia3 v3.2.0, Minigraph v0.20-r559, minimap2 v2.20 & v2.24, MMseq v2, Novosort v3.09.01, Orthofinder v2.5.5, PASA v2.4.1, Plink2 v2.00a3.3LM, SAMtools v1.16.1, smartpca v7.2.1, StringTie v2.1.5, STAR v2.7.8a, SyRI v1.6, TransDecoder v5.5.0, TRITEX pipeline (no version), UCLUST v11, vg toolkit v1.46.0 mosdepth v0.2.6, LTRharvest, LTRdigest, genometools, version 1.5.10, tRNAscan-SE-1.3, PGGB version 0.4.0, ODGI version 0.7.3, Bandage version 0.7.3

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 the sequence data collected in this study have been deposited at the European Nucleotide Archive (ENA) under BioProjects PRJEB40587, PRJEB57567 and PRJEB58554 (raw data for pangenome assemblies), PRJEB64639 (pan-transcriptome Illumina data), PRJEB64637 (transcriptome Isoseq data), PRJEB53924 (Illumina resequencing data), PRJEB45466-511 (raw data for gene space assemblies), PRJEB65284 (srh1 transcriptome data). Accession codes for individual genotypes are listed in supplementary tables: Supplementary Table 1 (pangenome assemblies and associated raw data), Supplementary Table 2 (transcriptome data), Supplementary Table 5 (Illumina resequencing), Supplementary Table 6 (gene space assemblies).

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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	*Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data exclusions	*Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	No blinding was done.

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

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	Seeds of the core1000 and pangenome panel are available from German federal ex situ genebank at IPK Gatersleben.de
Novel plant genotypes	We performed cas9-editing in cv. Golden Promise. Experimental details are given in the Online Methods, section "Cas9-mediated mutagenesis". We constructed a FIND-IT library in cv. 'Etincel' (6-row winter malting barley; SECOBRA Recherches). The FIND-IT 'Etincel' library was screened as described in Knudsen et al. 87 using a single assay for the isolation of srh1P63S variant [ID# CB-FINDit-Hv-014].
Authentication	Mutants were Sanger-sequenced to confirm the presence of mutational events. Mutants were grown in the greenhouse to evaluate rachilla phenotypes.