nature portfolio

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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 was used for data collection

Data analysis	PacBio HiFi reads were assembled using hifiasm53 v0.19.8 in Hi-C-integration mode using corresponding Hi-C reads with default parameters. HiFi reads were aligned to the assembly using minimap254 v2.26 and coverage was assessed using the pileup.sh tool from bbmap v39.01 (https://sourceforge.net/projects/bbmap/).Mitochondrial and contaminant contigs were identified using BLASTn55 and removed from the assemblies. The NuclearPhaser v1.1 pipeline was used to assess phasing (https://github.com/JanaSperschneider/NuclearPhaser). Scaffolding was performed using information from SALSA56 v2.2, Hi-C-Pro57 v3.1.0, Hicexplorer58, and synteny to hap01 from isolate Pgt21-0 (GenBank accession GCA_008522505.1) assessed by alignment with D-GENIES59. BUSCO360 v3.0.2 was used to assess completeness of the assemblies (-l basidiomycota_odb9). Telomeres were detected using the findtelomeres.py script. Gene and repeat annotation relied on , RepeatModeler v2.0.2a (-LTRStruct) and RepeatMasker v4.1.2p1 were run to model and mask repeats in the genomes (http://www.repeatmasker.org). RNAseq reads were aligned to their respective genomes with HISAT261 v2.2.1 and transcripts assembled with genome-guided Trinity62 v2.13.2 and Stringtie63 v2.2.1. CodingQuarry64 v2.0 was run in pathogen mode on the transcripts. We then ran funannotate65 v1.8.5 predict and supplied Trinity transcripts, CodingQuarry predictions, and Pucciniomycotina EST clusters from JGI MycoCosm (http://genome.jgi.doe.gov/pucciniomycotina/pucciniomycotina.info.html). To capture additional genes encoding secreted proteins, we ran TransDecoder v5.5.0 (https://github.com/TransDecoder/TransDecoder) on the StringTie infection transcripts and selected complete ORFs encoding proteins with a signal peptide predicted by SignalP66 v4.1 and no transmembrane domains predicted by TMHMM67 v2.0. Novel secreted protein encoding genes were added to the annotation unless they were >90% contained in a repetitive region.Whole genome alignment was performed with MUMmer68 v4.0.0rc1 (default nucmer parameters), and sequence divergence was assessed with dnadiffRecombination analysis was performed using the pangenome-graph approach using cactus71 v2.9.0. Mash72 v2.0 was used to measure k-mer containment of sketched reference haplotype-phased sequences (-s 100000) in genomic Illumina reads from Pgt isolates.

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 raw sequence data are available under NCBI BioProject PRJNA1267768. Assembly and annotation files are deposited in the CSIRO data access portal (https://data.csiro.au/collection/csiro:65828).

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	Sample sizes varied and are described in legends of figures or within figures. Three biological replicates performed for protoplast transformation experiments as is standard for the field. For the Nicotiana transformation experiments a minimum of six replicates were performed as standard for the field.
Data exclusions	no data were excluded.
Replication	Library screening experiments were performed twice with similar results. Protoplast and Nicotiana transformation experiments repeated at

	least twice with consistent results.
Randomization	not relevant to study as no subjects were allocated to experimental groups.
Blinding	not relevant to the study

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

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	no animals involved, the study involves rust (Puccinia graminis f. sp. tritici)
Wild animals	wild animals not involved
Reporting on sex	not applicable
Field-collected samples	no field samples collected
Ethics oversight	no ethical approval required as study did not involve human or animal subjects.

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

Plants

Seed stocks	wheat cultivar McNair and other cultivars representing stem rust differential set
Novel plant genotypes	not applicable
Authentication	not applicable