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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 to collect the phenotype data
Data analysis	Genome assembly and validation: hifiasm v0.19.5-r587; fastp v0.23.2; BWA mem v0.7.17-r1188; YaHS v1.2a.1; Juicebox v1.11 (https://github.com/aidenlab/Juicebox); LTR_FINDER_parallel v1.2; LTR_retriever; Merqury v1.3; BUSCO (poales_odb10 dataset). Gene model prediction: Liftoff; fastp v0.23.2; HISAT2 v2.2.1; Stringtie v2.2.1; pbmm2 v1.10.0; cDNA_Cupcake; TransDecoder v5.5.0; CD-HIT v.4.8.1; BUSCO v1.7.131; eggNOG-mapper v2.1.1232. Comparative synteny and SNP density assays: JCVI; fastp v0.23.2; BWA-mem v0.7.17-r1188; GATK; Freebayes v1.3.6; BCFtools v1.14. Candidate gene identification: STAR; SAMtools v1.8; Freebayes v1.3.6; BCFtools v1.14. Genome-specific primers: Primer 3 v0.4.0; Phylogenetic analysis: MEGA v7.0; iTOL v5.0 (https://itol.embl.de/); NCBI (ttps://www.ncbi.nlm.nih.gov); Reference genomes (https://wheat.pw.usda.gov/blast/); Structure prediction: AlphaFold v2, AlphaFold-Multimer, and ChimeraX.

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

Data supporting the findings of this work are available within the paper and its supplementary information files. All raw sequencing data, genome assembly, and gene annotation for this project are archived at the National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, under BioProject accession number PRJCA036461. The sequence of the Lr30 gene was deposited in NCBI GenBank under accession number PV159345. 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	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	In our mapping experiments, we utilized 286 plants from the cross PI 192051 × Rusty, along with an additional 315 plants from the cross PI 192051 × m1. The analysis of these populations revealed a recombination-sparse region.
Data exclusions	No data were excluded.
Replication	All experiments were repeated at least twice and multiple biological replicates were used in all experiments.
Randomization	We randomized mutant lines and wild type controls when phenotyping as is normal practice. When phenotyping transgenic plants, 25 seeds were randomly chosen from each T1 family. It is not relevant to other experiments as no subjects were allocated to experimental groups.
Blinding	Blinding was performed when phenotyping the F2 plants / mutant lines / transgenic plants, qRT-PCR for determining transgene expression, and cytogenetic assays.

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	Commercial antibodies: Anti-GFP (Abcam, Cambridge, UK; Catalog No. ab290; Lot No. GR3431263-1; 1:2500 dilution); Goat Anti-Rabbit IgG-HRP (Abmart, Shanghai, China; Catalog No. M21002S; Lot No.334666; 1:10000 dilution).
Validation	Validation of commercial antibodies is provided on the manufacturer's website: Anti-GFP (https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab290.html); Goat Anti-Rabbit IgG-HRP (http://www.ab-mart.com.cn/page.aspx?node=%2062%20&id=%20980).

Plants

Seed stocks	Wheat accessions PI 192051, RL6049, PI 619381, Rusty, and others were obtained from the U.S. Department of Agriculture National Small Grains Collection.
Novel plant genotypes	Susceptible EMS mutants were induced using EMS mutagenesis in the PI 192051 background. The mutant population was treated with 250 mL solutions of EMS to create a diverse pool of genetic variants. Additionally, CRISPR/Cas9-based gene editing was used to generate targeted mutations, while transgenic plants and introgression lines were developed to further explore gene function and trait improvement. Detailed methodologies for these approaches are comprehensively described within the paper.
Authentication	Independent EMS-induced susceptible mutants, CRISPR/Cas9-based knockout plants, and transgenic lines were utilized for comprehensive analysis. All mutants and transgenic plants were verified through PCR amplification, Sanger sequencing, and qRT-PCR analysis.