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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   More than 5 samples, and generally more than 10 samples, were used in each experiment. Each experiment was replicated at least twice. Please see the figure legends and online methods. Minimal (or in some cases exact) sample sizes used are indicated in specific sections of online methods.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   For each experiment all attempts at replication were successful

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Replicated samples were allocated in groups on a random basis

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Assembly of the NGS sequence reads was performed using the HGAP workflow (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP) of the SMRT® Analysis v2.2.0 software (Pacific Biosciences). RNA-seq data was mapped to the wheat genomic sequence using Hisat2 v2.0.4 (https://ccb.jhu.edu/software/hisat2/index.shtml). The BAM file was imported into Geneious v8.1.5 (Biomatters Ltd.) and the gene models curated by producing gene coding sequence (CDS) annotations that matched the mapped RNA-seq data. To identify and construct pseudogene annotations, the curated exons and TGACv1 WGA WAK-like gene exon annotations extracted from BioMart on Ensembl Plants (http://plants.ensembl.org/index.html) were aligned to the reference using Lastz v7.0 in Geneious. The genomic sequence was then translated on all 6 frames and the resulting amino acid sequences subjected to a scan for Pfam domains using HMMER v3.1 (http://hmmer.org/) to assist in curation. The NGS (Illumina) paired-end reads from RNA-seq experiments were mapped to wheat genomic DNA contigs using Tophat2 v2.0.13 with a mate inner distance set to 300 (-r), a mate standard deviation to 300, no mismatches are allowed (-m 0 -N 0) and qualities are set to --solexa1.3-quals. Mapping results were processed using the Picard Tools suite v1.124 (https://broadinstitute.github.io/picard/), to accept only reads with a mapping quality above 30. The duplicates were removed with MarkDuplicates in Picard. The transcript assembly was performed using Cufflinks v2.2.1 using filtered mapped reads. All the assemblies were then merged using Cuffmerge in Cufflinks, and FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values were calculated using default parameters. Comparison of the different identified haplotypes was performed using Molecular Evolutionary Genetics Analysis (MEGA) v5.10 software. Statistical analyses were done using GenStat (2016, 18th edition, VSN International, Hemel Hempstead, UK).

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	All unique materials used are readily available from the authors

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	An anti-Stb6 kinase domain peptide (sequence – DVQSGSSTRSEETSL) antiserum was custom produced at Eurogentec (Seraing, Belgium) and its specificity tested using a dot blot at 1:1000 dilution vs 200 ng peptide.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used in this study
b. Describe the method of cell line authentication used.	n/a
c. Report whether the cell lines were tested for mycoplasma contamination.	n/a
d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	n/a

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in this study

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants