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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	SSC were measured by a digital refractometer (PAL-1, ATAGO, JPN) Saccharide content was measured by UPLC-MS/MS (ACQUITY UPLC I-Class-Xevo TQ-S Micro, Waters) Bio-Rad CFX96 with CFX Maestro 1.1 software was used to qPCR analysis The fluorescence signal was detected using a confocal microscopy (Leica SP8) The LUC activity was analyzed using the Night SHADE LB985 (Berthold) and Tanon-5200 image system The Net photosynthesis rate was measured with the LICOR-6800 XT instrument
Data analysis	Image analysis: ImageJ (version 1.45) Statistical analysis: GraphPad Prism (version 8.00) Phylogenetic analysis: MEGA (version 11.0.10), hmmer (version 3.4), MAFFT (version 7.525), ggmsa (version 3.19) Saccharide content analysis: MassLynx V4.1 (Waters) Genetic statistics analysis: IQTREE (version 2.1.4), VCFtools (version 0.1.16), PLINK (version 1.90b6.21), ggplot2 (version 3.4.4)

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 data are available within this Article and its Supplementary Information (Supplementary Tables 1-10). Original uncropped gel and blot images are provided in Supplementary Figs. 1-2. Original data points in graphs are shown in the Source Data files. HMM models were downloaded from the InterPro database (https://www.ebi.ac.uk/interpro/entry/pfam). 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	The sex and gender information of the participants for each of the sensory evaluation panel has not been collected.
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	In the sensory evaluation study, approx. 100 participants (age 20- to 59-years-old) were selected for each of the sensory test. These participants were required to be healthy and without any known oral diseases.
Recruitment	All participants were previously informed as to the sources of the genome-edited tomato materials and the sensory procedure and requirement, and signed informed consent forms before the sensory tests. The participants for each of the sensory test were recruited randomly, without any self-selection bias, except that the participants with any known oral diseases were excluded, because they may be insensitive to the sweetness. Participants are not limited by sex or gender, both males and females could participate in the sensory tests.
Ethics oversight	The sensory test followed the Declaration of Helsinki, and the experimental protocol was approved by the Ethical Committee of Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences.

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 methods were used to determine sample size. The sample size is described in the relevant figure legends and supplementary information. The 402 accessions were selected to calculate the allele frequency based on our previous study (Tieman et al., Science 2017, 355, 391-394). For agronomic traits in the field, sample sizes were chosen based on previous publications (Song et al., Nature 2023, 617: 118-124; Liu et al., Nature 2021, 590: 600-605; Tian et al., Science 2019, 365: 658-664).
Data exclusions	The plants, which were diseased or grown in the guard rows, were marked and were excluded from the analyses.
Replication	Three biological replicates with three technical replicates were used in the qRT-PCR experiment. For the subcellular localization, histological analysis, Y2H, LCI and cell-free protein degradation assays, the results are representative of three independent experiments. Three independent transgenic knock-out lines were generated for SlCDPK27. All of these experiments were successfully repeated.
Randomization	All samples were arranged randomly into experimental groups.
Blinding	For molecular biology experiments, bias could not be introduced since samples were treated identically and collected randomly. The investigation of agronomic traits was performed without prior knowledge of the results, blind was also not applied. For the sensory evaluation of sweetness assay, each sample was labeled with random codes, and the paired samples were presented to the sensory assessors, for sensory evaluation, in a balanced order.

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

Anti-His (MBL, Cat# D291-3, 1:3,000 dilution)
Anti-Actin (Sigma, Cat# A0480, 1:10,000 dilution)
Horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) (ZSGB-BIO, Cat#ZB-2305, 1:10,000 dilution)
Horseradish Peroxidase-conjugated goat anti-rabbit IgG (H+L) (ZSGB-BIO, Cat#ZB-2301, 1:10,000 dilution)
Anti-SlCDPK27 (custom-developed by Shanghai Youke Biotechnology Co., Ltd, 1:100 dilution for IP-MS assay, 1:2,000 dilution for western blot assay)

Validation

Validation statements and experiments can be obtained from the following websites and publications:
Anti-His (https://www.mblbio.com/bio/g/dtl/A/?pcd=D291-3)
Anti-Actin (https://www.sigmaaldrich.com/CG/en/product/sigma/a0480)
Horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) (http://www.zsbio.com/product/ZB-2305)
Horseradish Peroxidase-conjugated goat anti-rabbit IgG (H+L) (http://www.zsbio.com/product/ZB-2301)
Residues 6-20 of SlCDPK27 was used to make antibodies by Shanghai Youke Biotechnology Co., Ltd, and S. lycopersicum Money Maker (MM) and SlCDPK27-CR2 were used to validate anti-SlCDPK27 antibody.

Plants

Seed stocks

The detailed information of all the 402 accessions used in this study are listed in Supplementary Table 8.

Novel plant genotypes

The SlCDPK27 and SlCDPK26 mutants with 6-bp or 5-bp deletions were generated by CRISPR/Cas9 system in S. lycopersicum Money Maker (MM) or the processing variety M82.

Authentication

The SlCDPK27 and SlCDPK26 mutants were verified by PCR and sequencing. All experiments were performed using homozygous lines without T-DNA integration. And all of the potential off-target sites for both SlCDPK27 and SlCDPK26, were tested by PCR and sequencing.