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<br>*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<br>*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 | Olympus FV3000-IX83, Carl Zeiss LSM800 (Confocal microscopy); JEOL JEM-1400FLASH (Electron microscopy imaging); nanoflow LC, timsTOF HT Bruker, EASY-nLC 1200 system, Thermo Fusion Lumos mass spectrometer (LC-MS/MS analysis); Bio-Rad CFX96 Real-Time system (RT-qPCR); Tanon Chemi Dog ULTRA imaging system, Tanon VE 680 vertical transfer system (Immunoblot assays); Nano Prometheus Panta instrument (Dynamic light scattering analysis); Fida Biosystems ApS (Flow-induced dispersion analysis); FEI Titan Krios (Cryo-EM imaging). |
| Data analysis | GraphPad Prism v.10.1.2, Microsoft Excel 2016 (Statistical analysis); SnapGene v6.0.2 (sequence analysis); Fiji-imagej (Fluorescence intensity analysis); ImageJ 1.51j8 (Electron microscopy imaging); Proteome Discoverer 2.5, DIA-NN (Mass spectrometry analysis); CFX Manager v3.1.1517.0823 (RT-qPCR); FIDA V3.1 (Flow-induced dispersion analysis); cryoSPARC v4.5.1 (cryo-EM single particles processing); Phenix version 1.16-3549-000 (structure refinement); Coot version 0.9.4.1 (structure model building); PyMOL version 3.1.6.1, UCSF Chimera version 1.16, UCSF ChimeraX version 1.5rc202211220112 (structure analysis); AlphaFold version 2.3.1 (structure modeling). PowerPoint 2016, Adobe Photoshop 2023, FigDraw 2.0 (https://www.figdraw.com/#/) (scientific illustration). |

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

Source data are provided with this paper. The cryo-EM maps of the Zea mays ZmGLN1 have been deposited in the Electron Microscopy Data Bank (https://www.emdataresource.org/EMD-66135) under the accession numbers EMD-66135. The atomic coordinate data of the ZmGLN1 have been deposited in the Protein Data Bank (https://www.rcsb.org/structure/unreleased/9WP6) under the accession numbers 9WP6. The proteomic and IP-MS data have been deposited in the ProteomeXchange Consortium via the iProX partner repository (https://www.iprox.cn/) under the accession numbers PXD068249 and PXD068233. The RNA-seq data of maize inbred lines and teosinte have been deposited in the NCBI database (https://www.ncbi.nlm.nih.gov/) under the BioProject IDs PRJNA1332834 and PRJNA1417018. All source data underlying the main and extended data figures are provided as separate Excel files in the Supplementary Information. The uncropped, raw images of all gels and blots are provided in Supplementary Fig. 4. All other data supporting the findings of this study are available within the article and its supplementary information files.

## 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 | No statistical methods were used to predetermine sample size. Sample size was estimated based on preliminary experiments and previously published results. We made sufficient samples to reproducibly observe statistically significant difference, sample size was stated in each panel. |
| Data exclusions | No data was excluded from the analyses. |
| Replication | All experiments in this study were repeated independently at least three times. This information is shown in fiqure legends. |
| Randomization | All samples were arranged randomly into experimental groups. Plants of equal initial sizes were randomly assigned to the treatment and control groups for test. |
| Blinding | For molecular biology experiments such as qRT-PCR and Western blot, the sample processing procedures were completely consistent. The operator was aware of group allocation during instrument loading, but data acquisition was performed automatically by the instruments and analyzed using objective algorithms without any manual interpretation. Multiple independent replicate experiments were conducted and yielded consistent results.<br>For transmission electron microscopy (TEM) observation and subcellular localization experiments, the phenotypic differences among treatment groups were visually very distinct. Nevertheless, samples were re-coded by a person not involved in the experiment, fields of view were randomly selected by the imager, and the statistician was blinded to group allocation during data analysis, thereby effectively controlling experimental bias.<br>For plant material cultivation, different nitrogen concentrations resulted in obvious phenotypic differences, making blinding impossible. But, when different genotypes were planted, they were randomly coded and could not be distinguished during the entire growth period; all plants |

received identical water and fertilizer treatment. Measured traits (plant height, fresh weight) were directly recorded by a single researcher and did not involve subjective interpretation.

# 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-FLAG (1:5000, Abclonal, clone AMC0382-HRP, RRID AB_2769864, cat. AE024),
Anti-Actin (1:5000, Abmart, clone 26F7, RRID AB_2936239, cat. M20009),
Anti-His (1:8000, Bioss, clone 6E6, RRID AB_3712315, cat. bsm-33004M),
Anti-HA (1:5000, Abmart, clone 26D11, RRID AB_2864345, cat. M20003),
Anti-PEPC (1:8000, Orizyems, polyclonal, cat. PAB230901),
Anti-Rubisco (1:8000, Abmart, clone 1A11,cat.M20043),
Anti-VTE1 (1:5000, Orizyems, polyclonal, cat. PAB210901),
Anti-TOC75 (1:5000, Orizyems, polyclonal, cat. PAB220823),
Anti-LHCB1 (1:8000, Orizyems, polyclonal, cat. PAB06001),
Anti-UGPase (1:5000, Orizyems, polyclonal, cat. PAB230718),
Anti-GLN1 (1:5000, Agrisera, polyclonal, RRID AB_1271078, cat. AS08 296),
Anti-Rabbit IgG-HRP (1:10000, Abmart, RRID AB_2713951, cat. M21002L),
Anti-Mouse IgG-HRP (1:10000, Abmart, RRID AB_2713950, cat. M21001L).

Validation

Antibody validation. All primary antibodies were selected based on manufacturer validation for the indicated species and applications. Validation documentation, including species, specificity testing, expected molecular weight, and application suitability, is available on the respective manufacturer product webpages listed below.

Anti-FLAG (Abclonal, clone AMC0382-HRP, RRID AB_2769864, cat. AE024, https://abclonal.com.cn./catalog/AE024). Meanwhile in this study, specific bands were detected in the NIR-overexpressing plants carrying the FLAG tag but were not detected in the wild type WT (Extended Data Fig.17).
Anti-Actin (Abmart, clone 26F7, RRID AB_2936239, cat. M20009, https://www.ab-mart.com.cn/page.aspx?node=%2059%20&id=%20985),
Anti-His (Bioss, clone 6E6, RRID AB_3712315, cat. bsm-33004M, https://www.biosschina.com/productDetail?goods_id=818),
Anti-HA (Abmart, clone 26D11, RRID AB_2864345, cat. M20003, https://www.ab-mart.com.cn/page.aspx?node=%2059%20&id=%20963),
Anti-PEPC (Orizyems, polyclonal, cat. PAB230901, https://phytoantibodies.com/3472.html),
Anti-Rubisco (Abmart, clone 1A11,cat.M20043, https://www.ab-mart.com.cn/page.aspx?node=%2059%20&id=%2017738),
Anti-VTE1 (Orizyems, polyclonal, cat. PAB210901, https://phytoantibodies.com/3819.html),
Anti-TOC75 (Orizyems, polyclonal, cat. PAB220823, https://phytoantibodies.com/3684.html),
Anti-LHCB1 (Orizyems, polyclonal, cat. PAB06001, https://phytoantibodies.com/3221.html),
Anti-UGPase (Orizyems, polyclonal, cat. PAB230718, https://phytoantibodies.com/3419.html),
Anti-GLN1 (Agrisera, polyclonal, RRID AB_1271078, cat. AS08 296, https://www.agrisera.com/en/artiklar/glutamine-synthetase-gln-gln-chloroplastic.html).

## 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 | The mutants nir2-1, nir2-2, gln2, gln3, gln4, gln5 and gln6 seeds were obtained from the mutant library (http://maizeems.qlnu.edu.cn/) and generated in the WT (B73 background). The ZmNIR2T1OE and ZmNIR2T2OE overexpression lines were generated in the WT (B73 background). The mutants nir1 and gln1 were created by Center for Crop Functional Genomics and Molecular Breeding of China Agricultural University and generated in the CK (ND101 background). The ZmNIR2T1OE-12 and ZmNIR2T1OE-23 overexpression lines were generated in the WT (YH7 background). The inbred lines and teosinte accessions by our own research team. |
| Novel plant genotypes | We constructed the gene into a vector driven by the UBI promoter and fused with a C-terminal FLAG tag. Using Agrobacterium tumefaciens strain EHA105 as the infection agent, we transformed the maize recipient lines B73 or YH7 to obtain T0 generation plants. After screening with specific PCR identification primers, at least 15 positive seedlings were selected and self-pollinated to produce T1 generation plants. Continuous self-pollination and PCR identification were carried out over multiple generations until homozygous positive stable T3 generation plants were obtained for subsequent experiments. This study generated overexpression lines of ZmNIR2T1OE and ZmNIR2T2OE in the B73 background, as well as the ZmNIR2T1OE-12 and ZmNIR2T1OE-23 overexpression lines in the YH7 background, using the methods described above. |
| Authentication | The plant materials were identified using PCR amplification in conjunction with next-generation sequencing technology. The mutation positions and patterns are shown in Extended Data Fig. 6, and the corresponding primers are listed in the primer list. |