nature portfolio

Corresponding author(s):	Felix Willmund
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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For a	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	\square 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.
\boxtimes	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	\boxtimes 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.
Sof	ftware and code

Policy information about availability of computer code

Data collection Collection

Collection was conducted with Microsoft Excel and Origin, Figures were assembled with Illustrator

Data analysis

Microscopy images were analyzed by the ZEN blue 2.3 or Fiji software and Lightbox 2024; Proteomics were analysed with Perseus

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 <u>policy</u>

Source data are provided with this paper. Proteomics data are available via ProteomeExchange consortium (http://www.ebi.ac.uk/pride) with the following identifier:

PXD057252 Data Set 1 - Global proteome of Col-0 and tig2 mutant

Research inv	olving hu	man participants, their data, or biological material			
,		vith human participants or human data. See also policy information about sex, gender (identity/presentation), thnicity 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 informa	ation on the appr	oval of the study protocol must also be provided in the manuscript.			
Field-spe	ecific re	porting			
Please select the or	ne below that is	s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
Life sciences	В	ehavioural & social sciences			
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces stu	udy design			
All studies must dis	close on these	points even when the disclosure is negative.			
Sample size	In general 3-4 biological replicates (individual plants from different pots) were collected. In addition, technical replicates (e.g. > 30 seedlings for size determination or multiple leaves with randomized sections for photosynthesis measurements) were measured.				
Data exclusions	For proteomic analyses, peptide counts were excluded if they were found in less than 3 o the 4 biological replicates. For all other experiments no data were excluded				
Replication	For proteomic analyses, 4 biological replicates (plants from different pots) were used. For Immunoblot three independent experiments were performed. Microscopy was performed for multiple leaves from several plants (>3) per condition and line.				
Randomization	nization Plant growth and harvesting was randomized to exclude positional effects during growth.				
Blinding	N/A				
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 & exp	perimental s	ystems Methods			
n/a Involved in the study n/a Involved in					
Antibodies		ChIP-seq			
	Eukaryotic cell lines				
	Palaeontology and archaeology MRI-based neuroimaging Animals and other organisms				
Clinical data					
Dual use research of concern					
☐ ☐ Plants	☐ ☑ Plants				

All gene accession numbers are given in the Supplementary Data and are according to the Arabidopsis standard https://www.arabidopsis.org.

PXD057264 Data Set 2 – Analysis of sedimented ribosomes

Antibodies

Antibodies used

All antibodies are listed in Supplemental Table S3. uL1c, Tig1, Tig2, RbcL are from own production. PsaA, PsbA, PsbC are from Agrisera. uL12c is a gift from RD Rochaix, GFP is purchased from Roche.

Validation

Commercially antibodies passed validation. Own antibody were tested against their epitope and in respective mutants (for lacking signal) if applicable. uL1, Tig1 and RbcL antibodies were published earlier (references are given in the manuscript)

Plants

Seed stocks

Seeds were obtained from the Nottingham Arabidopsis Stock Centre

Novel plant genotypes

Double mutant was generated by crossing.

Complementing lines and lines with tagged GFP were transformed into Agrobacterium tumefaciens (GV3101 containing pMP90)

Authentication

All lines were examined by PCR over the respective locus and sequencing. In addition immoblots and proteomics confirmed deletions