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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Proteome Discoverer (Version 1.4) developed by Thermo Scientific was used in LCMS workflow for peptide identification.
Data analysis	R (Version 3.5.3) was used for statistical analysis and graphics. R is open source and a free software environment. DESeq2 and DEP packages in R were used for proteomic analyses. MEGA (Version 7.0) software was used for protein sequence analysis from different species and is publicly available. Gene Ontology enrichment analysis was performed using the online tool OmicShare (http://omicshare.com/tools). Prediction of the transmembrane domain was performed using the SMART database (http://smart.embl-heidelberg.de/). The microarray-based gene expression data were downloaded from ATTED-II (http://atted.jp/).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All the mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (Identifier: PXD015919). MS data set names are listed in Supplementary Table 10. Those data are publicly available for download. Figures that have associated MS raw data include Fig.1, Fig.2, Fig.4, and Extended Data Fig.3. The microarray-based gene expression data were downloaded from ATTED-II (http://atted.jp/).

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	Due to the large amount of mass spectrometry experiments performed in this study, it is not possible to follow the sample size suggested by a typical Power Analysis. Nevertheless, for each MS profiling of each sample (genotype/treatment/cellular_fraction), we used three replicates, which is more than what is typically used in MS. A pilot study or the actual result itself is normally the most straightforward way to gauge whether the sample size is sufficient. For subtractive proteomics, the resulting NE proteome covers almost all the known nucleoporins and over 70% of know NE proteins is a clear indication of sufficient sample size used in this case (Fig.1). For proximity-labeling proteomics, we used Nup93a-BioID2 for a pilot assay (Fig.2). The fact that we identified 14 nucleoporins in the top 15 candidates of Nup93-interacting proteins is a evident support for the sufficient size of sample we used. We used 30 siliques to measure the lethal seed segregation ratio and 15 siliques for silique length measurement. These sample sized are sufficient to clearly reflect the 1:3 segregation ratio for lethal embryos in seed pods and the complementation of pnet1 hos1 phenotype by the PNET1-YFP transgene. Mutant genotypes in Fig. 4e-f were confirmed by PCR in nup160 pnet1+/- and hos1 pnet1+/- segregating progenies (n = 60 seedlings).
Data exclusions	No data were excluded from the analyses.
Replication	All fluorescent imaging and immunoblot data were successfully replicated and representative results were shown. The mass spectrometry experiments were replicated three times. Analyses were performed by incorporating data from all three times to obtain statistical inference of candidate. The three independent batches of sample included in each MS profiling usually displayed high levels of consistency. For lethal seed segregation ratio measurement, three replicates were used (each contain 10 siliques).
Randomization	For proximity-labeling proteomic experiments, samples were grouped according to genotypes and treatments. Each sample consists of 0.4 g randomly selected and pooled Arabidopsis seedlings of certain genotype with particular treatment (+/- 50 uM biotin). For subtractive proteomics, samples were grouped according to cellular fractions (nucleus or microsomal membrane). Each sample that was subject to cellular fractionation consists of 20g randomly selected and pooled wild-type Arabidopsis seedlings. For fluorescent microscopy, cells that express target proteins were randomly selected for imaging and fluorescence quantification. For seed set segregation, siliques were randomly sampled from 10 plants.
Blinding	This study did not involve paired tests, and blinding is not relevant.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-HA (clone 12CA5, Roche, Cat#11666606001), Anti-Actin (Abiocode, Cat#R3772-1P), Anti-Histone H3 (Abcam, Cat#ab18521)
Validation	Anti-HA (12CA5 is a mouse monoclonal antibody to a peptide epitope derived from the hemagglutinin protein of human influenza virus. It is used for the immunochemical detection of native influenza hemagglutinin protein and recombinant "epitope tagged" proteins containing the HA epitope in western and dot blots, immuno- cytochemistry, and immunoprecipitation.) We used 1:3000 dilution for western blot. Anti-Actin (R3772-1P is a rabbit polyclonal antibody to a fragment of the N-terminal region of Arabidopsis Actin-1 protein and is

suitable for western blot.) We used 1:3000 dilution for western blot.
Anti-Histone H3 (ab18521 is a rabbit polyclonal antibody to histone H3. It recognizes unmodified and modified forms of histone H3 and is suitable for western blot and immunoprecipitation. The antibody reacts with Cow, Human, Saccharomyces cerevisiae, and Xenopus laevis, and is predicted to work with Mouse, Arabidopsis thaliana, Caenorhabditis elegans, and Drosophila melanogaster). We used 1:3000 dilution for western blot.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	This study did not involve laboratory animals.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	No ethical approval or guidance is required for this study, which uses Arabidopsis thaliana as the model organism.

Note that full information on the approval of the study protocol must also be provided in the manuscript.