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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	No software was used.
Data analysis	cutadapt (v2.10) and trimmomatic (v0.36) were used to trim raw fastq files; HISAT2 (v2.1.0), HT-seq (v0.13.5), samtools (v1.10), DESeq2 (v3.14) were used in RNA-seq analysis. Bowtie2 (v2.2.9) and MACS2 (v2.2.7.1) were used in ChAP-seq analysis; EXCEL 2016 was used in two-sided Student's t-test; R (v4.0.4) was used in Tukey's HSD test; MINTmap (v1.0) was used in RIP-seq and sRNA-seq to identify and quantify tRFs and tRNA halves. FASTA (v36.3) was used to calculate sequence homology; bedtools (v2.26.0) was used to calculate the distance between ChAP-peaks and nearby genes; LTRharvest (v1.5.11), LTRfinder (v1.07) and LTR_retriever (v2.7) were used to identify LTR-retrotransposons; RNAfold (v2.4.18) was used to predict RNA secondary structure.

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

Data Availability Statement: Deep-sequencing data have been deposited in the SRA database and processed data from the ChAP-seq (bed alignment files, bw genome browser files, and narrowPeak peak-calling files) have been deposited to the GEO database. These SRA and GEO data are accessible through BioProject number PRJNA788635; a reviewer link has been created for the BioProject:https://dataview.ncbi.nlm.nih.gov/object/PRJNA788635?reviewer=r91943h51u70k10shli44uafvj. Processed data at the final step are provided as Extended Data Tables. All the data, the main text, or the supplementary materials are available from the corresponding author on request. 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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	The sample size was not predetermined by a statistical method, rather chosen as practically large as possible, following the common practice in the field. All the experiments involve 3 or more biological replicates, except for the RIP-seq with 2. In this RIP-seq, a single biological sample contains more than 20 individual plants to provide enough material for IP.
Data exclusions	No data were excluded.
Replication	Each experiment was repliably reproduced at least three times on separate occasions.
Randomization	All samples were assigned randomly into experimental groups.
Blinding	We were not blinded to allocation during experiments and their analysis. Instead, multiple people independently reproduced the outcomes that were essential for the conclusion in the MS. In addition, all the NGS experiments, generating sequencing data and its analysis were independently performed.

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

Goat polyclonal anti-HA-HRP (Novus, NB600-362), Rabbit polyclonal anti-Histone H3 (Abcam, ab1791), Rabbit polyclonal anti-phospho enol pyruvate carboxylase (Rockland, 200-4163), anti-DIG antibody (Roche, 11093274910) and Goat anti-Rabbit IgG H&L _HRP (Abcam, ab97051)

Validation

In addition to the manufacturers' validations, we independently validated all the antibodies against proteins/peptides by using proper negative and positive controls. Also, the immunoprecipitation specificity of anti-HA-HRP was further enhanced by using additional competition step in which HA peptides were used to release HA tagged proteins.

Plants

Seed stocks

All Arabidopsis lines used in this study are in the Col-0 background as a wild-type. The following mutant lines were obtained from the Arabidopsis Stock Center or the research groups in indicated references: ago1-27, ago2-1 (SALK_003380), ago3-2 (SALK_005335), ago4-2, ago5-1 (SALK_063806), ago6-3 (SALK_106607), ago7, ago8-1 (SALK_139894), ago9-2 (SALK_112059), ago10-2 (SALK_047336), ago2/3/710, dcl1-7, dcl2-1 (SALK_064627), dcl3-1 (SALK_005512), dcl4-2 (GABI_160G05), dcl2/3/4 (CS16391), HA-AGO1, HA-AGO2 and HA-AGO2-DAD. ago1/5/10 was generated from ago1-27, ago5-1, and ago10-2. HA-AGO1 (pAGO1::3HA-AGO1 in ago1-25), HA-AGO2 (pAGO2::3HA-AGO2 in ago2-1) and HA-AGO2-DAD (pAGO2::3HA-AGO2-DAD in ago2-1) lines are transgenic Arabidopsis carrying HA-tagged AGO1/AGO2 under their own promoters. tRF31Asp2_STTM and tRF31Asp2_mSS_STTM transgenic Arabidopsis, respectively carrying pFGC5941-PacI-STTM-tRF31Asp2_STTM and pFGC5941-PacI-STTM-tRF31Asp2_mSS_STTM, were generated in this study.

Novel plant genotypes

n/a

Authentication

All genotypes have been verified in previous studies as well as us and are cited in the corresponding manuscript.