

Corresponding author(s): Myriam Calo	onie	oni	Calc	am (Mvri):	(s	author	ponding	Corresp
--------------------------------------	------	-----	------	------	------	----	----	--------	---------	---------

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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Cor	nfirmed
	\boxtimes	The $\underline{\text{exact sample size}}(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
	\boxtimes	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
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on <u>statistics for biologists</u> may be useful.

Software and code

Policy information about availability of computer code

Data collection

H2A.Z ChIP seq data were collected from Gene Expression Omnibus (GEO) with accession number GSE96834 (Wollmann, H. et al., 2017) and H3K27me3 and H2AK121ub ChIP seq data form GEO under accession GSE89358 (Zhou, Romero-Campero et al., 2017)

Data analysis

Sequencing of RNA libraries was carried out with the Illumina HiSeq 2500 sequencer. The high quality of each sample was verified using the software package FASTQC. Read mapping to the A. thaliana TAIR10 reference genome and transcript assembly were performed with the software tools HISAT2 and StringTie. Differentially expressed genes (DEGs) were selected using the Bioconductor R packages Ballgown and LIMMA. Gene expression was measured in FPKM (fragments per kilobase of exon and million mapped reads). Differentially expressed genes were selected according to a log2-fold change cut-off>|1| in the different comparisons and a pvalue <0.05. ChIP-seq data for the localization of H3K27me3 in WT seedlings at 7 DAG were generated and analyzed previously (Zhou, Romero-Campero et al., 2017). For H2A.Z localization in WT seedlings at 10 DAG we used previously published data (Wollmann et al. 2017); accesion number GSE96834). Read mapping to the TAIR10 reference genome was performed using bowtie and peak calling was carried out with the software package MACS2. Metagene plots were generated with a custom R script based on the R Bioconductor packages ChIPeakAnno and Arabidopsis thaliana genome database R package, TxDb.Athaliana.Biomart.plantsmart2843. Processing of the BAM files were performed using the software package BEDTools to obtain RPKM normalized data. The area under the curve (AUC) was calculated using RPKM values at the defined regions of the gene (from TSS to 200 bp downstream TSS for +1 nucleosome region and from TSS to TES for entire gene length) with the zoo R package. H2A.Z signal was estimated by summing AUC of each gene.

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 upon request. 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 materials from this study are freely available from the authors. The RNA-seq datasets generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession GSE117969.

Field-spe	ecific reporting
Please select the b	est 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 <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>
Life scier	nces study design
All studies must dis	sclose on these points even when the disclosure is negative.
Sample size	For flowering time analyses, we used at least 20 plants of each genotype. For qRT-PCR analyses, we used n=4 biological replicates of each genotype. For each replicate RNA was extracted from 10 seedlings at 12 DAG. For Western blot quantifications, we used n=3 to 4 biological replicates of each genotype. For each replicate, Histone enriched extracts were obtained from 1 gr of seedlings at 7 DAG. For RNA-seq analyses, we used n=2 biological replicates of each genotype. For each replicate 5 mgr. of RNA was used for the analysis. For ChIP-qPCR analyses, we used n=2 to 4 biological replicates of each genotype. For each replicate chromatin was extracted from 1 gr. of seedlings at 7 DAG.
Data exclusions	We did not apply Data exclusion.
Replication	Experimental findings were reliably reproduced as indicated by the error bars of independent biological replicates.
Randomization	A. thaliana plants of different genotype were randomized across 1-2 trays in growth chamber conditions.
Blinding	We did not apply blinding group allocation during sampling.

Reporting for specific materials, systems and methods

Materials & experimental systems	Methods
n/a Involved in the study	n/a Involved in the study
Unique biological materials	ChIP-seq
Antibodies	Flow cytometry
Eukaryotic cell lines	MRI-based neuroimaging
Palaeontology	
Animals and other organisms	
Human research participants	

Antibodies

Antibodies used

The following primary antibodies were used: anti-HTA9 (agrisera AS10 718), anti-H4 (Abcam ab10158), anti-H3K27me3 (Diagenode, C15410069) anti-AtH2AK121ub (generated in the laboratory (Yang et al., 2013); anti-ubiqitin (Santa Cruz sc-8017) and anti-H3 antibody (abcam, ab1791)

Validation

anti-HTA9 (agrisera AS10 718), specific for Arabidopsis HTA9 (https://www.agrisera.com/en/artiklar/hta9-probable-histone-h2avariant-3.html) anti-H4 (Abcam ab10158), Validated for ChIP in Arabidopsis (Pacinka et al., 2010).

anti-H3K27me3 (Diagenode, C15410069), Validated for WB and ChIP in Arabidopsis (https://www.diagenode.com/en/p/h3k27me3-polyclonal-antibody-classic-50-mg-34-ml).

anti-AtH2AK121ub, generated and validated for WB in the laboratory (Yang et al., 2013)

anti-ubiqitin (Santa Cruz sc-8017), validated for Arabidopsis in the laboratory (Bratzel et al., 2010).

anti-H3 antibody (abcam, ab1791), validated for Arabidopsis (https://www.abcam.com/histone-h3-antibody-nuclear-loading-control-and-chip-grade-ab1791.html?productWallTab=Abreviews).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

We did not use eukaryotic cell lines in our study. We used Arabidopsis TDNA insertion lines which were obtained from public stock centers and previously characterized or generated new TDNA lines by the floral-dip method for this work.

Authentication

We confirmed the T-DNA insertion lines by locus specific PCR using genomic DNA as template, by gene expression and/or protein expression.

Mycoplasma contamination

Not applicable

Commonly misidentified lines (See <u>ICLAC</u> register)

Not applicable