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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 our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	x	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	x	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.
	x	A description of all covariates tested
	x	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 Give <i>P</i> values as exact values whenever suitable.
	x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	x	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	x	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	'	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used for data collection.

Data analysis

Quality control was performed using FASTQC v0.11.9 and Trimmomatic v0.39. Read mapping to the reference genome was carried out using HISAT2 v2.2.0 for RNA-seq data and bowtie2 v2.4.1 for ChIP-seq and ATAC-seq data. Transcript assembly and gene expression estimation was performed using StringTie v2.1.1. Differential Gene Expression analysis were carried out with the Bioconductor R packages Ballgown v2.20.0 and limma v3.44.3. GO term enrichment analysis were performed using the Bioconductor R packages clusterProfiler v3.16.0 and org.At.tair.db 3.11.4. Peakcalling and THS identification were carried out using MACS2 v2.2.6. BED file intersection and union were performed using bedtools v2.28.0. BAM file conversion was carried out with samtools v1.10. PCR Duplicates from ChIP-seq and ATAC-seq data were removed using Picard v2.21.0. BW files were generated using deepTools v3.3.2. Peaks and THS annotation were performed using the Bioconductor R packages ChIPpeakAnno v3.22.2 and ChIPSeeker v1.24.0. Several custom R scripts were used to generated signal profiles that are deposited in the GitHub repository https://github.com/fran-romero-campero/Rmetageneplots. Principal components analysis was performed using the R package FactoMiner v2.3.

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 Research guidelines for submitting code & software for further information.

Data

Blinding

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

Data supporting the findings of this work are available within the paper and its Supplementary Information files. The datasets and plant materials generated and analyzed during the current study are available from the corresponding author upon request. ATAC-seq, ChIP-seq and RNA-seq data sets generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession GSE155378 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155378). Previously generated ChIP-seq and RNA-seq data are under accession GSE89358. The source data underlying Figure 3, Figures 5, as well as Supplementary Figures 4, and 10 are provided as a Source Data file.

rieia-spe	ecific 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.
x Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of	f the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf
Life scie	nces study design
All studies must d	isclose on these points even when the disclosure is negative.
Sample size	We used 2 grams of 10-day-old seedlings from the different genotypes for ChIP-seq and ATAC-seq based on the published method, and 0.1 gram of 10-day-old seedlings for RNA-seq based on the RNA extraction kit instruction. We analyzed two biological replicates of each genotype for ATAC-seq and ChIP-seq and three for RNA-seq, which according to ATAC-seq Guidelines of Harvard FAS Informatics, and ChIP-seq and RNA-seq guidelines and practices of the ENCODE project are appropriated (see: https://informatics.fas.harvard.edu/atac-seq-guidelines.html https://www.encodeproject.org/documents/ceb172ef-7474-4cd6-bfd2-5e8e6e38592e/@@download/attachment/ChIP-seq_ENCODE3_v3.0.pdf https://www.encodeproject.org/documents/cede0cbe-d324-4ce7-ace4-f0c3eddf5972/@@download/attachment/ENCODE%20Best% 20Practices%20for%20RNA_v2.pdf).
Data exclusions	No data was excluded from our analysis.
Replication	We performed two biological replicates for ATAC-seq and ChIP-seq and three for RNA-seq. All attempts at replication were successful.
Randomization	All the samples were classified based on the genotype, such as WT Col-O and different PcG mutants.

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.

In our study blinding was not relevant as it does not affect the behavior of WT or mutant plants as well as data analysis.

Materials & experimental systems	Methods		
n/a Involved in the study	n/a Involved in the study		
Antibodies	ChIP-seq		
x Eukaryotic cell lines	X Flow cytometry		
Palaeontology and archaeology	MRI-based neuroimaging		
X Animals and other organisms	•		
Human research participants			
X Clinical data			
Dual use research of concern			

Antibodies

Antibodies used

Anti-trimethyl-Histone H3 (Lys27) Antibody, Millipore, Cat: 07-449, Lot: 3146226. Ubiquityl-Histone H2A (Lys119) (D27C4) XP® Rabbit mAb, Cell Signaling Technology, Cat: 8242S, Lot: 6.

Validation

Anti-trimethyl-Histone H3 (Lys27), also known as Anti-H3K27me3, is a highly published Rabbit Polyclonal Antibody. This protein A purified antibody is dot blot tested for trimethylated lysine 27 specificity and validated in WB, ICC, IP.

Ubiquityl-Histone H2A (Lys119) (D27C4) XP® Rabbit mAb is validated for:

W-Western IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide

ChIP-seq

Data deposition

x Confirm that both raw and final processed data have been deposited in a public database such as GEO.

x Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155378

Files in database submission

ATAC-seq raw data: atacseq_col0_1_1.fq.gz atacseq_col0_1_2.fq.gz atacseq_col0_2_1.fq.gz atacseq_col0_2_2.fq.gz atacseq_bmi1abc_1_1.fq.gz atacseq_bmi1abc_1_2.fq.gz atacseq_bmi1abc_2_1.fq.gz atacseq_bmi1abc_2_2.fq.gz atacseq_clfswn_1_1.fq.gz atacseq_clfswn_1_2.fq.gz atacseq_clfswn_2_1.fq.gz atacseq_clfswn_2_2.fq.gz atacseq_emf1_1_1.fq.gz atacseq_emf1_1_2.fq.gz atacseq_emf1_2_1.fq.gz atacseq_emf1_2_2.fq.gz atacseq_lhp1_1_1.fq.gz atacseq_lhp1_1_2.fq.gz $atacseq_lhp1_2_1.fq.gz$ atacseq_lhp1_2_2.fq.gz atacseq_ring1ab_1_1.fq.gz atacseq_ring1ab_1_2.fq.gz atacseq_ring1ab_2_1.fq.gz atacseq_ring1ab_2_2.fq.gz

ATAC-seq processed data: atacseq_col0_1.bw atacseq_col0_2.bw atacseq_bmi1abc_1.bw atacseq_bmi1abc_2.bw atacseq_clfswn_1.bw atacseq_clfswn_2.bw atacseq_emf1_1.bw atacseq_emf1_1.bw atacseq_lhp1_1.bw atacseq_lhp1_1.bw atacseq_lhp1_2.bw atacseq_ring1ab_1.bw atacseq_ring1ab_1.bw atacseq_ring1ab_1.bw atacseq_ring1ab_2.bw consensus ths.bed

ChIP-seq raw data: h2aub_col0_1_1.fq.gz h2aub_col0_1_2.fq.gz h2aub_col0_2_1.fq.gz h2aub_col0_2_2.fq.gz

```
h2aub_emf1_1_1.fq.gz
h2aub_emf1_1_2.fq.gz
h2aub_emf1_2_1.fq.gz
h2aub_emf1_2_2.fq.gz
h2aub_ring1ab_1_1.fq.gz
h2aub_ring1ab_1_2.fq.gz
h2aub_ring1ab_2_1.fq.gz
h2aub_ring1ab_2_2.fq.gz
h3k27me3_col0_1_1.fq.gz
h3k27me3_col0_1_2.fq.gz
h3k27me3_col0_2_1.fq.gz
h3k27me3_col0_2_2.fq.gz
h3k27me3_emf1_1_1.fq.gz
h3k27me3_emf1_1_2.fq.gz
h3k27me3_emf1_2_1.fq.gz
h3k27me3_emf1_2_2.fq.gz
h3k27me3_ring1ab_1_1.fq.gz
h3k27me3_ring1ab_1_2.fq.gz
h3k27me3_ring1ab_2_1.fq.gz
h3k27me3_ring1ab_2_2.fq.gz
h3k27me3_lhp1_1_1.fq.gz
h3k27me3_lhp1_1_2.fq.gz
h3k27me3_lhp1_2_1.fq.gz
h3k27me3_lhp1_2_2.fq.gz
input_col0_1.fq.gz
input_col0_2.fq.gz
ChIP-seq processed data:
h2aub_col0_1.bw
h2aub_col0_2.bw
h2aub_emf1_1.bw
h2aub_emf1_2.bw
h2aub_ring1ab_1.bw
h2aub_ring1ab_2.bw
h3k27me3_col0_1.bw
h3k27me3_col0_2.bw
h3k27me3_emf1_1.bw
h3k27me3_emf1_2.bw
h3k27me3_ring1ab_1.bw
h3k27me3_ring1ab_2.bw
h3k27me3_lhp1_1.bw
h3k27me3 lhp1 2.bw
input col0.bw
h2aub consensus peaks.bed
h3k27me3_consensus_peaks.bed
RNA-seq raw data:
col0_1_1.fq.gz
col0_1_2.fq.gz
col0_2_1.fq.gz
col0_2_2.fq.gz
col0_3_1.fq.gz
col0_3_2.fq.gz
clfswn_1_1.fq.gz
clfswn_1_2.fq.gz
clfswn_2_1.fq.gz
clfswn_2_2.fq.gz
clfswn_3_1.fq.gz
clfswn_3_2.fq.gz
ring1ab_1_1.fq.gz
ring1ab_1_2.fq.gz
ring1ab_2_1.fq.gz
ring1ab_2_2.fq.gz
ring1ab\_3\_1.fq.gz
ring1ab\_3\_2.fq.gz
emf1\_1\_1.fq.gz
emf1\_1\_2.fq.gz
emf1_2_1.fq.gz
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emf1_2_2.fq.gz
emf1_3_1.fq.gz
emf1_3_2.fq.gz
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RNA-seq processed data: gene_expression.tsv

Genome browser session (e.g. <u>UCSC</u>)

http://genome.ucsc.edu/s/fran_rom_cam/Polycomb%20Accesibility

Methodology

Repl	icates
1 (CP	icates

For all ChIP-seq and ATAC-seq, we did two biological replicates; for RNA-seq, we did three biological replicates.

Sequencing depth

ATAC-seq:						
Col0 rep1	44,247,510	18.78%	paired-end	d 19	50nt	
Col0 rep2	47,788,632	18.80%	paired-end	d 15	50nt	
bmi1abc rep1	48,364,697	26.32%	paired-end		50nt	
bmi1abc rep2	39,317,062	26.03%	paired-end		50nt	
clfswn rep1	52,074,011	36.06%	paired-end		50nt	
clfswn rep2	32,689,404	28.35%	paired-end		50nt	
emf1 rep1	42,209,845	41.99%	paired-end		50nt	
emf1 rep2	39,814,391	40.43%	paired-end		50nt	
lhp1 rep1	46,171,107	18.71%	paired-end		50nt	
lhp1 rep2	48,995,300	19.12%	paired-end		50nt	
ring1ab rep1	53,087,907		paired-end		iOnt	
ring1ab rep1	49,457,730		paired-end		iOnt	
ııııgıan iehz	43,437,730	24./470	pan eu-enc	a 13	OHL	
ChIP-seq:						
Col0 H2Aub rej	p1	12,780,243	87.24%	paired	l-end	150nt
Col0 H2Aub rej	'	12,641,870	89.26%	paired-		150nt
emf1 H2Aub re	•	11,204,529	90.54%	paired		150nt
emf1 H2Aub re	•	13,164,450	90.90%	paired		150nt
ring1ab H2Aub	•	11,107,370	88.82%	paired		150nt
ring1ab H2Aub	'	11,948,572	86.16%	paired		150nt
Col0 H3K27me		20,207,084	74.83%	paired-		150nt
Col0 H3K27me		18,237,177	74.82%	paired-		150nt
emf1 H3K27me		21,269,223	73.33%	paired-		150nt
emf1 H3K27me		16,751,109	66.67%	paired-		150nt
ring1ab H3K27		18,151,696	79.75%	paired-		150nt
ring1ab H3K27		19,540,914	80.21%	paired-		150nt
lhp1 H3K27me		21,457,122	59.57%	paired		150nt
Ihp1 H3K27me		21,752,391	59.85%	paired		150nt
pr 113KZ/IIIE	5 1 CP2	_1,102,001	JJ.UJ/U	Pulleu	CIIU	130111
RNA-seq:						
Col0 rep1	20,226,42	98.30%	paired-e	nd	150nt	
Col0 rep2	21,042,08		paired-e		150nt	
bmi1abc rep1	54,627,44		paired-er		150nt	
bmi1abc rep2	46,742,56		paired-er		150nt	
clfswn rep1	14,890,45		paired-er		150nt	
clfswn rep2	15,636,97		paired-er		150nt	
emf1 rep1	20,527,83		paired-er		150nt	
emf1 rep2	19,557,34		paired-er		150nt	
ring1ab rep1	19,518,70		paired-er		150nt	
ring1ab rep1	22,409,40		paired-er		150nt	
01001002	22, 103,40	55.1770	pan ca ci		100110	

Antibodies

Anti-trimethyl-Histone H3 (Lys27) Antibody, Millipore, Cat: 07-449, Lot: 3146226. Ubiquityl-Histone H2A (Lys119) (D27C4) XP® Rabbit mAb, Cell Signaling Technology, Cat: 8242S, Lot: 6.

Peak calling parameters

 $bowtie 2 -p \ 5 -x \ \$INDEX -1 \ \$SAMPLE_LEFT -2 \ \$SAMPLE_RIGHT -S \ \$\{SAMPLE_NAME\}. samEnd \ And \ Anti-American \ Anti-Am$ $macs2\ callpeak\ -t\ \$\{SAMPLE_NAME\}.bam\ -c\ \$\{SAMPLE_INPUT\}\ -f\ BAM\ --outdir\ .\ -n\ \$\{_NAME\}$

Data quality

16310 consensus H2Aub peaks 4367 consensus H3K27me3 peaks

23226 consensus THS

Peaks were determined using default parameters independently for each replicate. Only peaks present in both replicates were considered.

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Software

MACS2

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