nature portfolio

Corresponding author(s):	Keqiang Wu
Last updated by author(s):	2022/08-04

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>.

o.					
٧t	2	ŀ١	C1	۲ı.	CS

1 01	ali statisticai ai	ialyses, commit that the following items are present in the figure regend, table regend, main text, or interious section.				
n/a	Confirmed					
	The exact	sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
	A stateme	ent 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 descript	tion of all covariates tested				
	A descript	tion 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.					
	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				
	ı	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.				
So	ftware an	d code				
Poli	cy information	about <u>availability of computer code</u>				
Da	ata collection	Only public software/codes were used.				
Da	ata analysis	Only public software/codes were used.				
		g 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				

Data

Policy information about <u>availability of data</u>

All manuscripts must include a <u>data availability statement</u>. 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

To review GEO accession GSE195735:

Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE195735

Enter token mxilkwmqflq	hxej into the box				
Human researd	ch participants				
	ut <u>studies involving human research participants and Sex and Gender in Research.</u>				
Reporting on sex and	Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in to source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.				
Population character	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."				
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.				
Ethics oversight	Identify the organization(s) that approved the study protocol.				
lote that full information	on the approval of the study protocol must also be provided in the manuscript.				
ife scienc	es study design				
Il studies must disclos	se on these points even when the disclosure is negative.				
Sample size 20-	-50 plants of each group				
Data exclusions no	data exclusion				
Replication	ee success biological replicates				
Randomization Use	e WT plant as control, and compared to mutant plants				
Blinding	Each sample were random selected.				
Reporting	for specific materials, systems and methods				
We require information fr	rom authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,				
ystem or method listed is	s 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 & exper	imental systems Methods				
n/a Involved in the st	n/a Involved in the study ChIP-seq				
	Eukaryotic cell lines Flow cytometry				
Palaeontology	cology and archaeology MRI-based neuroimaging				
Animals and ot	her organisms				
Clinical data	ech of concorn				
Dual use resear	un or concern				

Antibodies

Antibodies used

Anti-GFP (Santa Cruz Biotechnologies, catalog no. SC-9996; 1:3000 dilution) and anti-AS1 (SIGMA catalog no. M2; 1:3000 dilution) antibodies were used as primary antibodies for Western blot. Antibodies against anti-FLAG (SIGMA, catalog no. M2), H3Ac (Millipore, catalog no. 06-599) or H3K9me2 (diagenode, C15410060) were used for ChIP.

Validation

Anti-GFP (Santa Cruz Biotechnologies, catalog no. SC-9996) https://www.scbt.com/p/gfp-antibody-b-2?requestFrom=search anti-AS1(Luo et al., 2012) https://doi.org/10.1371/journal.pgen.1003114

anti-FLAG (SIGMA, catalog no. M2) https://www.sigmaaldrich.com/TW/en/product/sigma/f3165

 $H3Ac \ (Millipore, catalog \ no. \ 06-599) \ https://www.merckmillipore.com/JP/ja/product/Anti-acetyl-Histone-H3-2000 \ https://www.merckmillipore.com/JP/ja/product/Anti-acetyl-H3-2000 \ https://www.merckmillipore.com/JP/ja/product/A$

Antibody, MM NF-06-599

H3K9me2 (diagenode, C15410060) https://www.diagenode.com/en/p/h3k9me2-polyclonal-antibody-classic-50-ug-44-ul

ChIP-seq

Data deposition

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

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.

To review GEO accession GSE195735:

Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE195735

Enter token mxilkwmqflqhxej into the box

Files in database submission

GSM5849480 KYP:3xFLAG-IP-1 GSM5849481 KYP:3xFLAG-IP-2 GSM5849482 KYP:3xFLAG-input

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

To review GEO accession GSE195735:

Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE195735

Enter token mxilkwmqflqhxej into the box

Methodology

Replicates

Two independent KYPpro::KYP:3xFLAG/kyp transgenic lines were used as biological replicates for ChIP-seq experiment.

Sequencing depth

Approximately 24 and 16 million mapped reads of KYPpro::KYP:3xFLAG transgenic line #1 and #4 were used for analysis (pair-end, 150bp).

Antibodies

 $anti-FLAG \ (SIGMA, catalog \ no. \ M2) \ https://www.sigmaaldrich.com/TW/en/product/sigma/f3165$

Peak calling parameters

MACS2 were used for peak calling with default setting.

Data quality

3,924 genomic regions targeted by KYP were identified by MACS2.

Software

The alignments were first converted to Wiggle (WIG) files using deepTools. The data were then imported into the Integrated Genome Viewer (IGV) (Thorvaldsdottir et al., 2013) for visualization. The distribution of the ChIP binding peaks was analyzed with ChIPseeker (Table S2) (Yu et al., 2015), and a high-read random Arabidopsis genomic region subset (1,350,000 regions) was used to represent the ratio of the total Arabidopsis genomic regions. To identify DNA motifs enriched sites, 400-bp sequences encompassing each peak summit (200 bp upstream and 200 bp downstream) were extracted and searched for enriched DNA motifs using MEME-ChIP with the default parameters (Machanick and Bailey, 2011).