nature portfolio

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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 for data collection
Data analysis	Fastp version 0.20.1 Bowtie2 version 2.4.2 Picard version 2.24.0 MACS2 version 2.1.2 DeepTools BS-Seeker2 version 2.1.8 CGmaptools version 0.1.2 R version 1.1.1106 DESeq2 version 1.26.0 IRFinder version 1.3.1

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

The datasets generated in this study are available in the GEO: GSE274521, GSE274522, GSE274523, GSE274524, GSE274525, and GSE274526.

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	*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 in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if 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.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*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.*

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	No sample size calculation was performed
Data exclusions	No data exclusion
Replication	Three replications were performed for RT-qPCR, RNA-seq, and ChIP-qPCR. Two replications were performed for ChIP-seq, BS-seq, and ATAC-seq
Randomization	For all examples, samples were placed side by side
Blinding	No blinding needed

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
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[ ]	[x] Plants

Methods

n/a	Involved in the study
[ ]	[x] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

anti-GFP (Roche, 11814460001)
anti-GFP (Thermo Fisher Scientific, A11122)
anti-H2A.Z (Yelagandula et al., 2014)
anti-H3K4me1 (Abcam, ab8895)
anti-H3K4me2 (Abclonal, A22143)
anti-H3K4me3 (Abcam, ab8580)
anti-H3K27me3 (Millipore, 07-449)
anti-H3K36me3 (Abcam, ab9050)
anti-H3 (Abcam, ab1791)
anti-HA (CST, 3724)
anti-RNA Pol II Ser5p (Abcam, ab5131)
anti-RNA Pol II Ser2p (Abcam, ab5095)
anti-H3K27me1 (Millipore, 07-448)
anti-H2B (Abcam, ab1790)

Validation

anti-GFP (Roche, 11814460001): https://www.sigmaaldrich.com/US/en/product/roche/11814460001?srsltid=AfmBOoptKvj5rpLFetbnSQKUJCzttp2f8mt-Zwu_wyT1ZayVNEClHbpw
anti-GFP (Thermo Fisher Scientific, A11122): https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-11122
anti-H2A.Z (Yelagandula et al., 2014): doi: 10.1016/j.cell.2014.06.006
anti-H3K4me1 (Abcam, ab8895): https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895?srsltid=AfmBOooGwJBVDPCK1dYerFyxUCvU0wP0aLHVV_mIs6w4Qs1uzKWtBOeT
anti-H3K4me2 (Abclonal, A22143): https://eutemp.abclonal.com/catalog-antibodies/DiMethylHistoneH3K4RabbitmAb/A22143
anti-H3K4me3 (Abcam, ab8580): https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580?srsltid=AfmBOooiexKTrhd-ilSr7VOOVufgKoVHElAVKjoAXU2wcsPMU-YTPVDV
anti-H3K27me3 (Millipore, 07-449): https://www.merckmillipore.com/IE/en/product/Anti-trimethyl-Histone-H3-Lys27-Antibody,MM_NF-07-449?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
anti-H3K36me3 (Abcam, ab9050): https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k36-antibody-chip-grade-ab9050?srsltid=AfmBOorZaquLlDoRWkLpK615LGo23fCRVAQ2VpwU3iJEPCdAolOGvvxj
anti-H3 (Abcam, ab1791): https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-antibody-nuclear-marker-and-chip-grade-ab1791?srsltid=AfmBOoo6jARH3iVv-oOVTld68nHAamdNR3eAcaPFj95ic8rJRmwbYYIC
anti-HA (CST, 3724): https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724?srsltid=AfmBOoqHAQIn45nD5xppTrJCU8kWUHmTJJHMxRcICtSfOqHabwuNDM4E
anti-RNA Pol II Ser5p (Abcam, ab5131): https://www.abcam.com/en-us/products/primary-antibodies/rna-polymerase-ii-ctd-repeat-ysptsps-phospho-s5-antibody-ab5131?srsltid=AfmBOorlKJo89VaEOYvPpaN5x57jTzyiE0wvu1GlLx1OkalFEwsp9cy-
anti-RNA Pol II Ser2p (Abcam, ab5095): https://www.abcam.com/en-us/products/primary-antibodies/rna-polymerase-ii-ctd-repeat-ysptsps-phospho-s2-antibody-ab5095?srsltid=AfmBOoqlzifoby38J17dNBFcbmskQ8GpflhFVbo1AH-Rs2YoHjEU1KaA
anti-H3K27me1 (Millipore, 07-448): https://www.merckmillipore.com/IE/en/product/Anti-monomethyl-Histone-H3-Lys27-Antibody,MM_NF-07-448?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
anti-H2B (Abcam, ab1790): https://www.abcam.com/en-us/products/primary-antibodies/histone-h2b-antibody-chip-grade-ab1790?srsltid=AfmBOopB82o5mD3GSMnazR5Fs611HYhUA35ufw5LdzwPzNn5Fx9B60Lf

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
✘	☐	Public health
✘	☐	National security
✘	☐	Crops and/or livestock
✘	☐	Ecosystems
✘	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
✘	☐	Demonstrate how to render a vaccine ineffective
✘	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
✘	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
✘	☐	Increase transmissibility of a pathogen
✘	☐	Alter the host range of a pathogen
✘	☐	Enable evasion of diagnostic/detection modalities
✘	☐	Enable the weaponization of a biological agent or toxin
✘	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	h3.1kd (Jiang and Berger, 2017), h3.3ko (Zhao et al., 2022) and the sdg2 mutant (Salk_021008) (Guo et al., 2010) were reported previously.
Novel plant genotypes	h3.3ko;HTR5 K4A, transgenic, 3 lines, T3; h3.3ko;HTR5 K9A, transgenic, 1 line, T3; h3.3ko;HTR5 K27A, transgenic, 1 line, T3 h3.3ko;HTR5 T31A, transgenic, 1 line, T3; h3.3ko;HTR5 Y41F, transgenic, 1 line, T3; h3.3ko;HTR5 H87S, transgenic, 1 line, T3 h3.3ko;HTR5 L90A, transgenic, 1 line, T3; h3.3ko;HTR5 H87S&L90, transgenic, 2 line, T3; h3.3ko;HTR13, transgenic, 2 line, T3 h3.3ko;HTR5 R2A, transgenic, 2 line, T3; h3.3ko;HTR5 T3A, transgenic, 2 line, T3; h3.3ko;HTR5 T3E, transgenic, 2 line, T3 h3.3ko;HTR5 Q5A, transgenic, 2 line, T3; h3.3ko;HTR5 R8A, transgenic, 2 line, T3; h3.3ko;HTR5 T6E, transgenic, 2 line, T3 h3.3ko;HTR5 K4R, transgenic, 1 line, T3; h3.3ko;HTR5 K4Q, transgenic, 1 line, T3; Col;HTR5 T6A, transgenic, 148 line, T1 Col;HTR13 T6A, transgenic, 67 line, T1; Col;HTR5 H87S&L90A T6A, transgenic, 65 line, T1; HTR5-HA, transgenic, 2 lines, T3 HTR5 K4A-HA, transgenic, 2 lines, T3; HTR5 H87S&L90A-HA, transgenic, 2 lines, T3; HTR5 R2A-HA, transgenic, 2 lines, T3 HTR5 T3A-HA, transgenic, 2 lines, T3; HTR13-HA, transgenic, 2 lines, T3; HTR5-GFP, transgenic, 2 lines, T3 HTR5 K4A-GFP, transgenic, 2 lines, T3; HTR5 H87S-GFP, transgenic, 2 lines, T3; HTR5 L90A-GFP, transgenic, 2 lines, T3 HTR5 H87S&L90A-GFP, transgenic, 2 lines, T3
Authentication	PCR amplify the transgene or do sanger sequencing

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.*	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274524 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274525 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274526
Files in database submission	HTR5-GFP #1_Input HTR5-GFP #2_Input HTR5 K4A-GFP #1_Input HTR5 K4A-GFP #2_Input HTR5-GFP #1_IP HTR5-GFP #2_IP HTR5 K4A-GFP #1_IP

HTR5 K4A-GFP #2_IP
Col_Input_rep1
Col_Input_rep2
h3.3ko;HTR5_Input_rep1
h3.3ko;HTR5_Input_rep2
h3.3ko;HTR5 K4A_Input_rep1
h3.3ko;HTR5 K4A_Input_rep2
sdg2_Input_rep1
sdg2_Input_rep2
Col_H3K4me3_rep1
Col_H3K4me3_rep2
h3.3ko;HTR5_H3K4me3_rep1
h3.3ko;HTR5_H3K4me3_rep2
h3.3ko;HTR5 K4A_H3K4me3_rep1
h3.3ko;HTR5 K4A_H3K4me3_rep2
sdg2_H3K4me3_rep1
sdg2_H3K4me3_rep2
Col_Pol II Ser5p_rep1
Col_Pol II Ser5p_rep2
h3.3ko;HTR5_Pol II Ser5p_rep1
h3.3ko;HTR5_Pol II Ser5p_rep2
h3.3ko;HTR5 K4A_Pol II Ser5p_rep1
h3.3ko;HTR5 K4A_Pol II Ser5p_rep2
sdg2_Pol II Ser5p_rep1
sdg2_Pol II Ser5p_rep2
Col_Pol II Ser2p_rep1
Col_Pol II Ser2p_rep2
h3.3ko;HTR5_Pol II Ser2p_rep1
h3.3ko;HTR5_Pol II Ser2p_rep2
h3.3ko;HTR5 K4A_Pol II Ser2p_rep1
h3.3ko;HTR5 K4A_Pol II Ser2p_rep2
sdg2_Pol II Ser2p_rep1
sdg2_Pol II Ser2p_rep2

Genome browser session
(e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Two replicates were performed for each ChIP-seq experiment

Sequencing depth

All sequencing reads are paired-end 150bp, listed are the numbers of uniquely mapped reads
HTR5-GFP #1_Input: 31,895,185
HTR5-GFP #2_Input: 16,393,606
HTR5 K4A-GFP #1_Input: 30,342,906
HTR5 K4A-GFP #2_Input: 14,560,683
HTR5-GFP #1_IP: 14,180,734
HTR5-GFP #2_IP: 12,287,044
HTR5 K4A-GFP #1_IP: 22,042,004
HTR5 K4A-GFP #2_IP: 15,514,696
Col_Input_rep1: Arabidopsis_10,170,728, human_546,465
Col_Input_rep2: Arabidopsis_9,529,064, human_631,108
h3.3ko;HTR5_Input_rep1: Arabidopsis_14,169,073, human_569,411
h3.3ko;HTR5_Input_rep2: Arabidopsis_12,740,385, human_558,199
h3.3ko;HTR5 K4A_Input_rep1: Arabidopsis_13,325,922, human_536,930
h3.3ko;HTR5 K4A_Input_rep2: Arabidopsis_12,377,811, human_370,942
sdg2_Input_rep1: Arabidopsis_11,232,259, human_520,360
sdg2_Input_rep2: Arabidopsis_18,068,661, human_770,199
Col_H3K4me3_rep1: Arabidopsis_13,849,935, human_1,769,578
Col_H3K4me3_rep2: Arabidopsis_24,466,243, human_2,725,369
h3.3ko;HTR5_H3K4me3_rep1: Arabidopsis_15,856,888, human_1,310,466
h3.3ko;HTR5_H3K4me3_rep2: Arabidopsis_12,630,270, human_1,063,107
h3.3ko;HTR5 K4A_H3K4me3_rep1: Arabidopsis_13,364,359, human_2,026,839
h3.3ko;HTR5 K4A_H3K4me3_rep2: Arabidopsis_12,468,045, human_1,670,373
sdg2_H3K4me3_rep1: Arabidopsis_9,592,079, human_1,753,930
sdg2_H3K4me3_rep2: Arabidopsis_18,903,174, human_2,613,181
Col_Pol II Ser5p_rep1: 6,074,516
Col_Pol II Ser5p_rep2: 7,306,097
h3.3ko;HTR5_Pol II Ser5p_rep1: 9,733,397

h3.3ko;HTR5_Pol II Ser5p_rep2: 8,955,299
h3.3ko;HTR5 K4A_Pol II Ser5p_rep1: 9,829,602
h3.3ko;HTR5 K4A_Pol II Ser5p_rep2: 16,059,735
sdg2_Pol II Ser5p_rep1: 10,660,934
sdg2_Pol II Ser5p_rep2: 9,871,846
Col_Pol II Ser2p_rep1: 5,219,081
Col_Pol II Ser2p_rep2: 3,278,979
h3.3ko;HTR5_Pol II Ser2p_rep1: 6,732,661
h3.3ko;HTR5_Pol II Ser2p_rep2: 4,729,984
h3.3ko;HTR5 K4A_Pol II Ser2p_rep1: 5,624,488
h3.3ko;HTR5 K4A_Pol II Ser2p_rep2: 7,007,222
sdg2_Pol II Ser2p_rep1: 10,223,875
sdg2_Pol II Ser2p_rep2: 7,757,922

Antibodies

anti-GFP (Thermo Fisher Scientific, A11122), anti-H3K4me3 (Abcam, ab8580), anti-RNA Pol II Ser5p (Abcam, ab5131), anti-RNA Pol II Ser2p (Abcam, ab5095)

Peak calling parameters

system('cat call_peak.txt | while read id; do sample_name=${id%.re*}; ~/anaconda2/bin/macs2 callpeak -t $id -f BAMPE -g 1.2e8 -q 0.01 --max-gap 150 --call-summits -n ${sample_name} 2> ${sample_name}".log"; done')
system('cat call_peak.txt | while read id1 id2; do sample_name1=${id1%%.*}; sample_name2=${id2%%.*}; ~/anaconda2/bin/macs2 callpeak -t $id1 -c $id2 --broad --broad-cutoff 0.05 -f BAMPE -g 1.2e8 -n ${sample_name1}"vs"${sample_name2} 2> ${sample_name1}"vs"${sample_name2}".log"; done')

Data quality

Col_H3K4me3_rep1: 15741 peaks
Col_H3K4me3_rep2: 15683 peaks
h3.3ko;HTR5_H3K4me3_rep1: 15861 peaks
h3.3ko;HTR5_H3K4me3_rep2: 15979 peaks
h3.3ko;HTR5 K4A_H3K4me3_rep1: 16220 peaks
h3.3ko;HTR5 K4A_H3K4me3_rep2: 16264 peaks
sdg2_H3K4me3_rep1: 15160 peaks
sdg2_H3K4me3_rep2: 16812 peaks

Software

fastp version 0.20.1
Bowtie version 2.4.2
Picard version 2.24.0
SAMtools version 1.9
MACS2 version 2.1.2