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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 Authors & Referees 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	- Epson Scan 2 Perfection V600 (6.4.99) software (Epson) for serial dilution assay picture acquisition and western blotting image acquisition. - Metamorph software (v7) (Universal Imaging Corporation) for cytology acquisition.

Data analysis

- Fiji Image J (2.0)
- Snapgene 5.2
- FastQC (v0.11.9)
- FastQscreen (v0.14.1)
- Trimmomatic (v0.39)
- Cutadapt (v2.8)
- Bowtie2 (v2.3.5.1)
- Samtools (v1.10)
- STAR (v2.7.3)
- Salmon (v0.12.0)
- deepTools (v3.5.0)
- MACS2 (v2.2.7.1)
- IGV (v2.8.10)
- Bioconductor (R v4.1.1):
  - Sushi (v1.28.0)
  - DESeq2 (v.1.30.1)

-Metamorph (v7)
-MaxQuant (1.6.1.0)

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. 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

Data Availability
The publicly accessible PomBase database https://www.pombase.org was used to obtain genome sequence and annotation for the Schizosaccharomyces pombe (fission yeast) genome. All raw and processed reads from sequencing experiments (ChIP-seq and RNA-Seq) are available at GEO with accession number GSE190267. All raw mass spectrometry data are available at PRIDE using project accession number PXD030205.

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, the samples sizes used are considered standard in the field and were selected to ensure robust and statistically significant comparisons.

For ChIP-qPCR and RT-qPCR experiments, biological triplicates (samples independently cultured for the experiment) were used for each strain analysed

For RNA-seq experiments two biological replicates (samples independently cultured for the experiment) were performed for each strain analysed.

For ChIP-seq three biological replicates were performed for each strain and results were confirmed by ChIP-qPCR.

For experiments performed to test whether specific genetic mutant strains form more caffeine-resistant or fluconazole-resistant colonies than wild-type cells, 3 biological replicates were used to derive statistics. Equal volumes from a culture from each strain were plated on 8-10 (indicated in figure legend) caffeine/fluconazole-containing media plates. After 7 days, the number of resistant colonies on each plate was counted. Sample sizes are provided in the figure legends.

For quantification of cytological samples by microscopy, 100 cells for each sample was assayed.

Data exclusions

There were no data exclusions

Replication

Findings were reliably reproduced.
For ChIP-qPCR and RT-qPCR, data are mean +/- standard deviation from 3 biological replicates.

ChIP-seq experiments were performed three times and results were confirmed by ChIP-qPCR.
RNA-seq experiments were performed two times using two biological replicates.
Serial dilution growth assays were repeated at least twice on different days (biological replicates) with similar results.
Resistance frequency measurements were performed 3 times on distinct starting cultures (biological replicates).

Randomization

No randomization was required because the results of physical measurements of biomolecules, phenotypic analysis (e.g., drug resistance test) or sequencing of nucleic acid libraries are not affected by sample randomization.

Blinding

No blinding was required because the results of physical measurements of biomolecules, phenotypic analysis (e.g., drug resistance test), or sequencing of nucleic acid libraries are not affected by the researchers knowledge of sample identities.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

- Anti-Myc - Cell Signalling - 9B11 - for western analysis. 1:1000.
- Anti-FLAG M2 - Merck - F1804 - for western ananlysis 1:4000
- Anti-H3K9me2 - Mouse monoclonal 5.1.1 - for H3K9me2 ChIP-qPCR. Kindly provided by Takeshi Urano. 1 ug per ChIP-qPCR.
- Anti-GFP - Life Technologies - A11122 - for GFP ChIP-qPCR and cytology. 2 ug per ChIP-qPCR. 1:200 for cytology.
- Anti-GFP - Merck - 11814460001- for immunoprecipitation - 20 ug per IP. for western analysis 1:750
- Anti-alpha-tubulin - for western analysis. Kindly provided by Keith Gull. 1:15000.
- Anti-Bip1 - for western analysis. Lab stock. 1:1000.
- Anti-Cnp1 - for cytology. Lab stock 1:3000.
-peroxidase anti-peroxidase - Merck - P1291 -for western analysis. 1:1000.
- Anti-Mto2 - for western analysis. Kindly provided by Ken Sawin. 1:1000.
- Chicken anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 - for cytology - Life Technologies - A21441. 1:1000.
- Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 - for cytology - Life Technologies - A11016. 1:1000.
- Peroxidase conjugate goat anti-rabbit IgG - for western analysis - Merck - A6154. 1:10000
- Peroxidase conjugate goat anti-mouse IgG - for western analysis - Merck - A4416. 1:10000
- Anti-ubiquitin mouse monoclonal - for western analysis - Enzo - BML-PW8810 1:1000.

Validation

- Mouse mAb 5.1.1: Raised in Urano Lab, validated in Nakagawachi et al (2003) Oncogone 22, 8835. Additionally, this antibody has been validated in our lab using a strain lacking the H3K9 methyltransferase.
- Anti-alpha-tubulin raised in Gull lab, validated in Woods et al (1989) J. Cell. Sci. 93 (Pt 3), 491–500.
- Anti-Bip1 raised and validated in Pidoux & Armstrong (1993) J. Cell. Sci. 105 (Pt 4), 1115–1120.
- All other antibodies have been extensively used for ChIP, western and cytology analyses in our laboratory and have been validated using no tag (GFP/FLAG/Myc) controls. For previous studies where these antibodies have been used see Tong et al. (2019) Nat. Commun and Bayne et al. (2010) Cell.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All Schizosaccharomyces pombe strains used in this study are derivatives of 972 h- or other commonly used lab strains. Detailed genotypes are listed in Supplementary Table 1.

Name Genotype Source
972 h- wild type lab stock
A5885 h- lid2-TAP:ura4+ ura4-D18 leu1-32 ade6-210 his3D Parental FL201. Gift from Fei Li (Cande lab)
B2835 h- epe1-GFP-KAN Torres-Garcia, 2020
B3111 S.octosporus wild type Lab stock
B3204 h- epe1-13Myc-NAT This study
B3250 S.cerevisiae Sgo1-GFP Lab stock, gift from Adele Marston

B3826 h- 3xFLAG-epe1-GFP-KAN Torres-Garcia, 2020
B3827 h- 3xMyc-epe1-GFP-KAN This study
B3828 h- 100 amino acids deletion from N-terminus of epe1-GFP-KAN This study
B3829 h- 150 amino acids deletion from N-terminus of epe1-GFP-KAN This study
B3830 h- 200 amino acids deletion from N-terminus of epe1-GFP-KAN This study
B4001 h? Ade6P-sfGFP-Ura4-Terminator Lab stock
B4038 h? epe1-GFP-KAN mts2-1 TS This study
B4039 h+ epe1-13Myc-NAT mts2-1 TS This study
B4040 h-sty1Δ::NAT, epe1-GFP-KAN This study
B4288 h-gad8Δ::NAT epe1-GFP-KAN This study
B4289 h-pmk1Δ::NAT,epe1-GFP-KAN This study
B4292 h-atf1Δ::NAT, epe1-GFP-KAN This study
B4621 h- epe1 deletion by Crispr Torres-Garcia, 2020
B4782 h- 50 amino acids deletion from N-terminus of epe1-GFP-KAN This study
B4789 h-pek1Δ::NAT This study
B4940 h- 101-110 amino acids deletion from epe1-GFP-KAN This study
B4942 h- 111-120 amino acids deletion from epe1-GFP-KAN This study
B4944 h- 121-130 amino acids deletion from epe1-GFP-KAN This study
B5048 h-pmp1Δ::HYG,epe1-GFP-KAN This study
B5090 h-N-ter 150 amino acids of Epe1 with GFP This study
B5623 h- 151-170 amino acids deletion from epe1-GFP-KAN This study
B5625 h- 171-190 amino acids deletion from epe1-GFP-KAN This study
B5627 h- 190-210 amino acids deletion from epe1-GFP-KAN This study
B4057 h-clr4Δ::NAT This study
B5641 h- 100 amino acids deletion from N-terminus of epe1-GFP-KAN, clr4Δ::NAT This study
B5645 h- 150 amino acids deletion from N-terminus of epe1-GFP-KAN, clr4Δ::NAT This study
B5650 h- epe1 deletion by Crispr, clr4Δ::NAT This study
B5654 h- epe1-GFP-KAN, clr4Δ::NAT This study
B5775 h-hul5Δ::NAT This study
B5776 h-pub3Δ::NAT This study
B6243 h- Epe1-13Myc NAT, ddb1Δ::HYG This study
B6453 h- tEpe1-cd-GFP This study
B6455 h- Epe1-cd-GFP This study
B6469 h- hul5Δ::HYG, pub3Δ::NAT This study
B6483 h- tepe1-GFP resistant colony-1 This study
B6484 h- tepe1-GFP resistant colony-2 This study
B6485 h- tepe1-GFP resistant colony-3 This study
B6486 h- tepe1-GFP resistant colony-4 This study
B6487 h- tepe1-GFP resistant colony-5 This study
B6488 h- tepe1-GFP resistant colony-6 This study
B6489 h- tepe1-GFP resistant colony-7 This study
B6490 h- tepe1-GFP resistant colony-8 This study
B6491 h- tepe1-GFP resistant colony-9 This study
B6492 h- tepe1-GFP resistant colony-10 This study
B6493 h- tepe1-GFP untreated colony-1 This study
B6494 h- tepe1-GFP untreated colony-2 This study
B6495 h- tepe1-GFP untreated colony-3 This study
B6496 h- tepe1-GFP untreated colony-4 This study

Authentication

All strains were generated by transformation and combined using genetic crosses. Strains were verified by the presence or absence or marker genes allowing growth on selective medium and/or by PCR to determine the presence of the desired genetic alteration and/or by DNA sequencing and/or western blotting to confirm the presence of epitope tags, as appropriate.

Mycoplasma contamination

Not applicable - Yeast strains do not suffer from mycoplasma contamination, but all cultures were observed by microscopy to be free of bacterial contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used. This study used the yeast Schizosaccharomyces pombe.

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.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

epe1D_input_rep1_R1.fastq.gz
epe1D_input_rep2_R1.fastq.gz
epe1D_input_rep3_R1.fastq.gz

epe1D_input_rep4_R1.fastq.gz
Epe1GFP_input_rep1_R1.fastq.gz
Epe1GFP_input_rep2_R1.fastq.gz
Epe1GFP_input_rep3_R1.fastq.gz
Epe1GFP_input_rep4_R1.fastq.gz
Epe1DNP150GFP_input_rep1_R1.fastq.gz
Epe1DNP150GFP_input_rep2_R1.fastq.gz
Epe1DNP150GFP_input_rep3_R1.fastq.gz
Epe1DNP150GFP_input_rep4_R1.fastq.gz
epe1D_rep1_R1.fastq.gz
epe1D_rep2_R1.fastq.gz
Epe1GFP_rep1_R1.fastq.gz
Epe1GFP_rep2_R1.fastq.gz
Epe1DNP150GFP_rep1_R1.fastq.gz
Epe1DNP150GFP_rep2_R1.fastq.gz
epe1D_IP_rep1_R1.fastq.gz
epe1D_IP_rep2_R1.fastq.gz
epe1D_IP_rep3_R1.fastq.gz
epe1D_IP_rep4_R1.fastq.gz
Epe1GFP_IP_rep1_R1.fastq.gz
Epe1GFP_IP_rep2_R1.fastq.gz
Epe1GFP_IP_rep3_R1.fastq.gz
Epe1GFP_IP_rep4_R1.fastq.gz
Epe1DNP150GFP_IP_rep1_R1.fastq.gz
Epe1DNP150GFP_IP_rep2_R1.fastq.gz
Epe1DNP150GFP_IP_rep3_R1.fastq.gz
Epe1DNP150GFP_IP_rep4_R1.fastq.gz
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epe1D_input_rep4_R2.fastq.gz
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epe1D_IP_rep1_R2.fastq.gz
epe1D_IP_rep2_R1.fastq.gz
epe1D_IP_rep2_R2.fastq.gz
epe1D_IP_rep3_R1.fastq.gz
epe1D_IP_rep3_R2.fastq.gz
epe1D_IP_rep4_R1.fastq.gz
epe1D_IP_rep4_R2.fastq.gz
Epe1GFP_input_rep1_R1.fastq.gz
Epe1GFP_input_rep1_R2.fastq.gz
Epe1GFP_input_rep2_R1.fastq.gz
Epe1GFP_input_rep2_R2.fastq.gz
Epe1GFP_input_rep3_R1.fastq.gz
Epe1GFP_input_rep3_R2.fastq.gz
Epe1GFP_input_rep4_R1.fastq.gz
Epe1GFP_input_rep4_R2.fastq.gz
Epe1GFP_IP_rep1_R1.fastq.gz
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Epe1GFP_IP_rep3_R1.fastq.gz
Epe1GFP_IP_rep3_R2.fastq.gz
Epe1GFP_IP_rep4_R1.fastq.gz
Epe1GFP_IP_rep4_R2.fastq.gz
Epe1DNP150GFP_input_rep1_R1.fastq.gz
Epe1DNP150GFP_input_rep1_R2.fastq.gz
Epe1DNP150GFP_input_rep2_R1.fastq.gz
Epe1DNP150GFP_input_rep2_R2.fastq.gz
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Epe1DNP150GFP_input_rep4_R1.fastq.gz
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Epe1DNP150GFP_IP_rep2_R2.fastq.gz
Epe1DNP150GFP_IP_rep3_R1.fastq.gz
Epe1DNP150GFP_IP_rep3_R2.fastq.gz
Epe1DNP150GFP_IP_rep4_R1.fastq.gz
Epe1DNP150GFP_IP_rep4_R2.fastq.gz

epe1D_rep1_R1.fastq.gz
epe1D_rep1_R2.fastq.gz
epe1D_rep2_R1.fastq.gz
epe1D_rep2_R2.fastq.gz
Epe1GFP_rep1_R1.fastq.gz
Epe1GFP_rep1_R2.fastq.gz
Epe1GFP_rep2_R1.fastq.gz
Epe1GFP_rep2_R2.fastq.gz
Epe1DNP150GFP_rep1_R1.fastq.gz
Epe1DNP150GFP_rep1_R2.fastq.gz
Epe1DNP150GFP_rep2_R1.fastq.gz
Epe1DNP150GFP_rep2_R2.fastq.gz
epe1D_input_rep1_R1.fastq.gz
epe1D_input_rep2_R1.fastq.gz
epe1D_input_rep3_R1.fastq.gz
epe1D_input_rep4_R1.fastq.gz
epe1D_IP_rep1_R1.fastq.gz
epe1D_IP_rep2_R1.fastq.gz
epe1D_IP_rep3_R1.fastq.gz
epe1D_IP_rep4_R1.fastq.gz
Epe1GFP_input_rep1_R1.fastq.gz
Epe1GFP_input_rep2_R1.fastq.gz
Epe1GFP_input_rep3_R1.fastq.gz
Epe1GFP_input_rep4_R1.fastq.gz
Epe1GFP_IP_rep1_R1.fastq.gz
Epe1GFP_IP_rep2_R1.fastq.gz
Epe1GFP_IP_rep3_R1.fastq.gz
Epe1GFP_IP_rep4_R1.fastq.gz
Epe1DNP150GFP_input_rep1_R1.fastq.gz
Epe1DNP150GFP_input_rep2_R1.fastq.gz
Epe1DNP150GFP_input_rep3_R1.fastq.gz
Epe1DNP150GFP_input_rep4_R1.fastq.gz
Epe1DNP150GFP_IP_rep1_R1.fastq.gz
Epe1DNP150GFP_IP_rep2_R1.fastq.gz
Epe1DNP150GFP_IP_rep3_R1.fastq.gz
Epe1DNP150GFP_IP_rep4_R1.fastq.gz
epe1D_rep1_R1.fastq.gz
epe1D_rep2_R1.fastq.gz
Epe1GFP_rep1_R1.fastq.gz
Epe1GFP_rep2_R1.fastq.gz
Epe1DNP150GFP_rep1_R1.fastq.gz
Epe1DNP150GFP_rep2_R1.fastq.gz

Genome browser session
(e.g. UCSC)

Not applicable. Visualized data using IGV.

Methodology

Replicates

3 ChIP-seq replicates were performed for each strain. Results were confirmed by ChIP-qPCR.

Sequencing depth

All libraries were sequenced by 75 bp paired-end reads. We did not calculate number of uniquely mapped reads, since Bowtie2 default options do not do so. Heterochromatin regions/sequences are repetitive and the number of uniquely mapped reads is not informative.

Antibodies

Mouse mAb 5.1.1 anti-H3K9me2 for H3K9me2 ChIP-seq

Peak calling parameters

macs2 callpeak -f BAMPE -t sample.bam -c sample.bam --broad -g 14e6 --broad-cutoff 0.05 -n sample

Data quality

MACS2 was used to call peaks from paired-end ChIP-seq reads.

Software

- FastQC (v0.11.9)
- FastQscreen (v0.14.1)
- Trimmomatic (v0.39)
- Cutadapt (v2.8)
- Bowtie2 (v2.3.5.1)
- Samtools (v1.10)
- STAR (v2.7.3)
- Salmon (v0.12.0)
- deepTools (v3.5.0)
- MACS2 (v2.2.7.1)
- IGV (v2.8.10)
- Bioconductor (R v4.1.1):
  - Sushi (v1.28.0)
  - DESeq2 (v.1.30.1)