

## Supplementary Materials for **KDM5 lysine demethylases are involved in maintenance of 3'UTR length**

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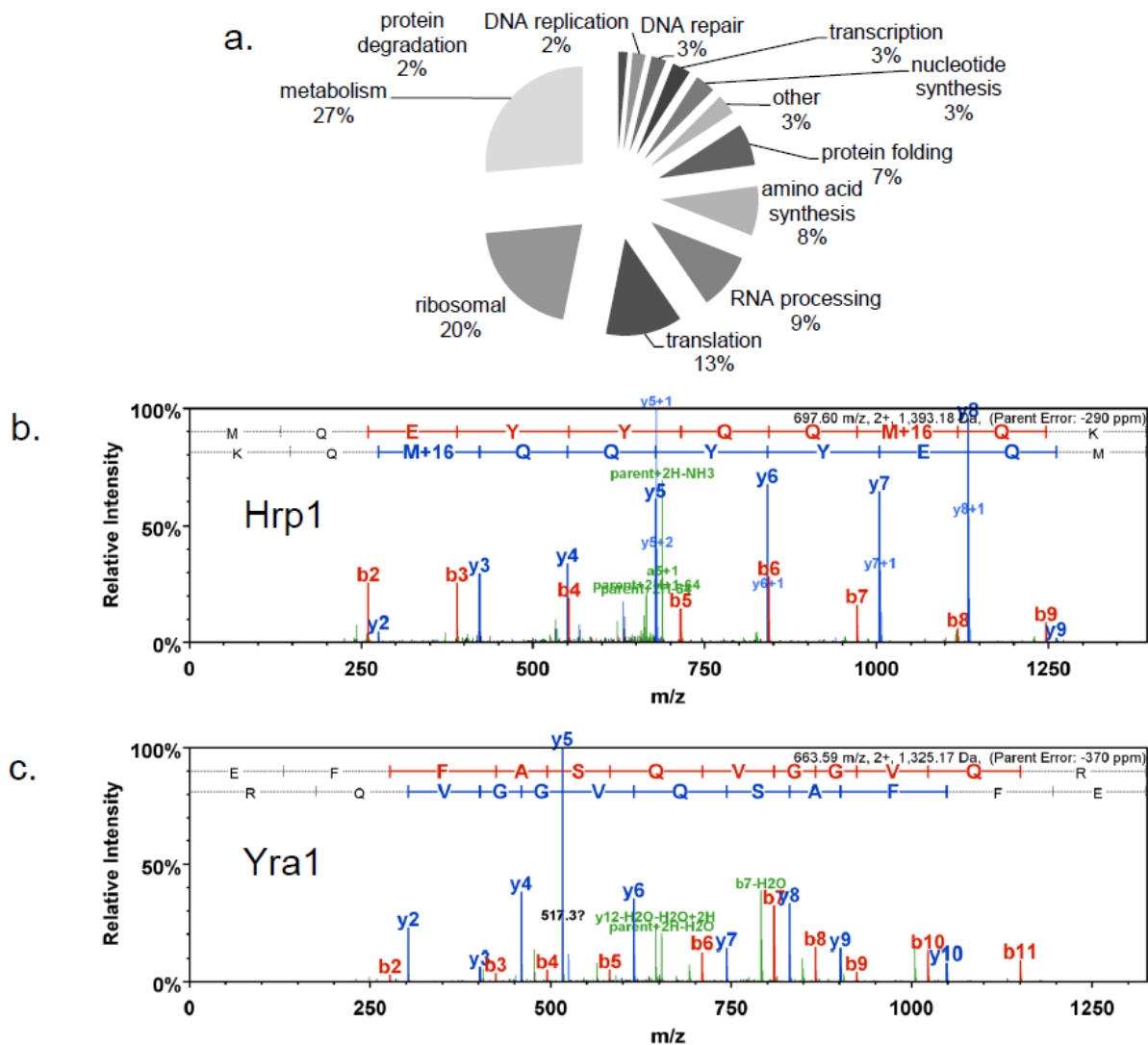
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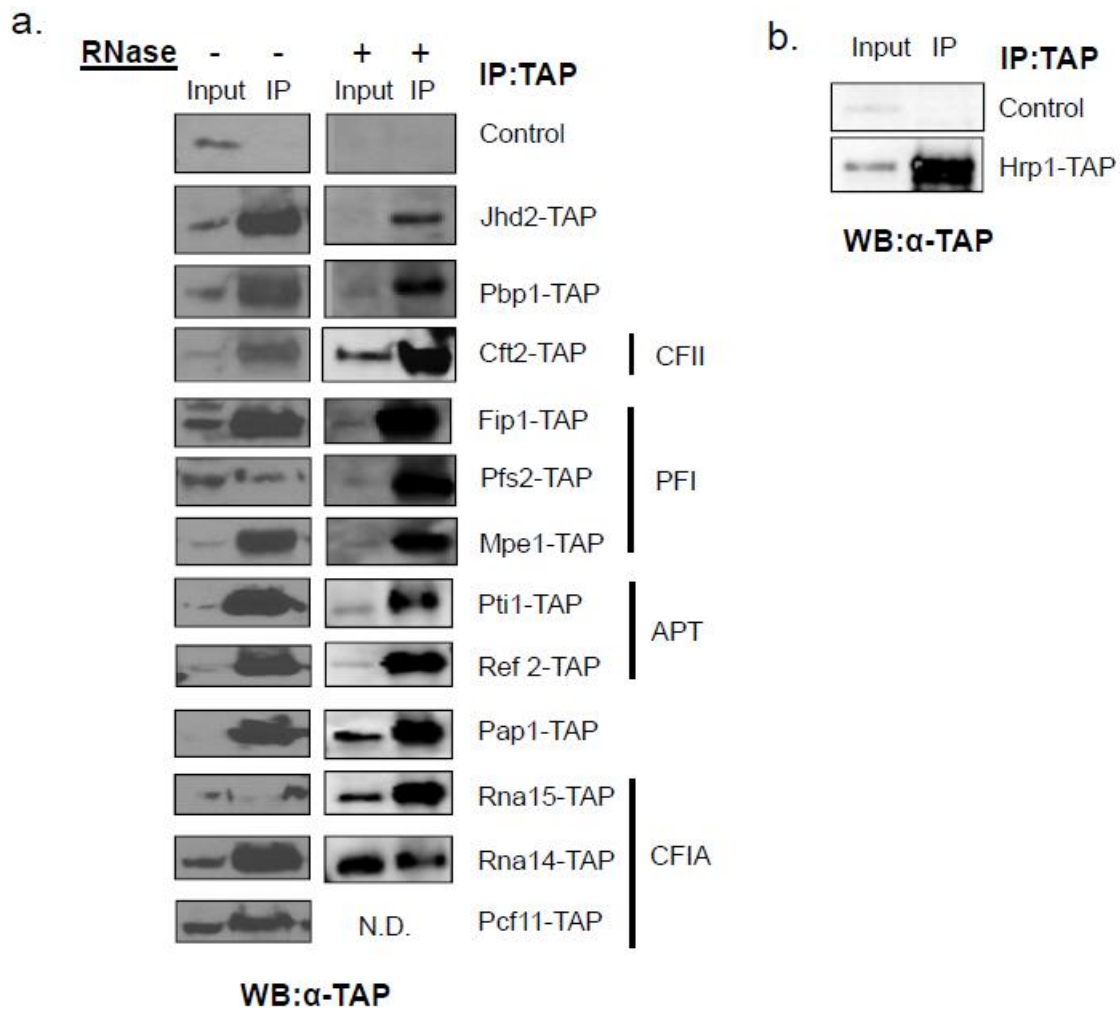
### **Other Supplementary Material for this manuscript includes the following:**

(available at [advances.sciencemag.org/cgi/content/full/2/11/e1501662/DC1](http://advances.sciencemag.org/cgi/content/full/2/11/e1501662/DC1))

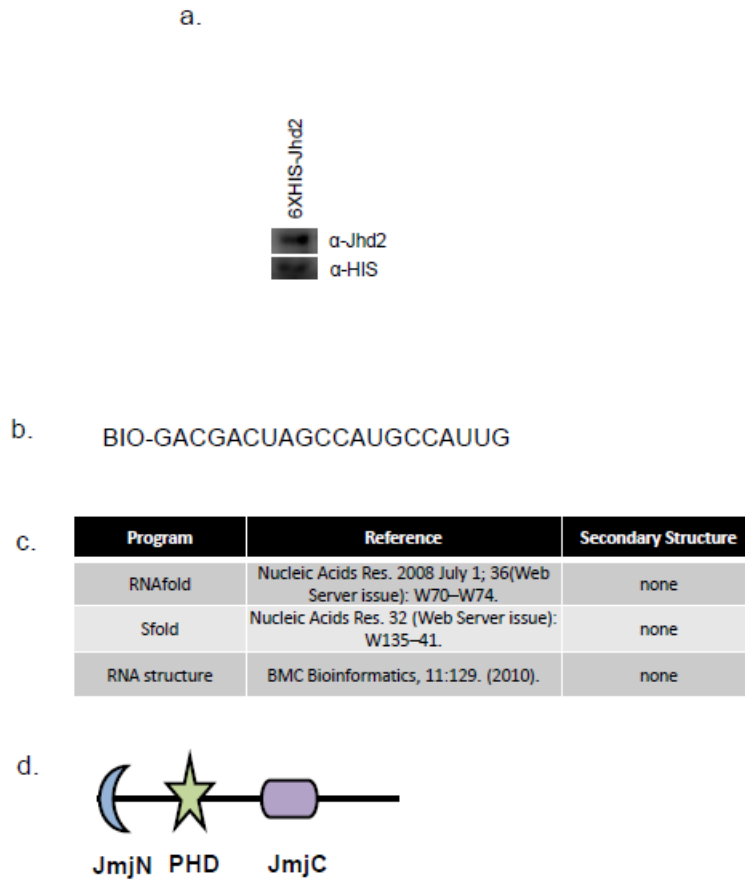
- table S1 (Microsoft Excel format). Jhd2 bound peaks identified using SICER.
- table S2 (Microsoft Excel format). Pcf11 bound peaks identified using SICER.
- table S3 (Microsoft Excel format). List of genes with APA after Jhd2 deletion.



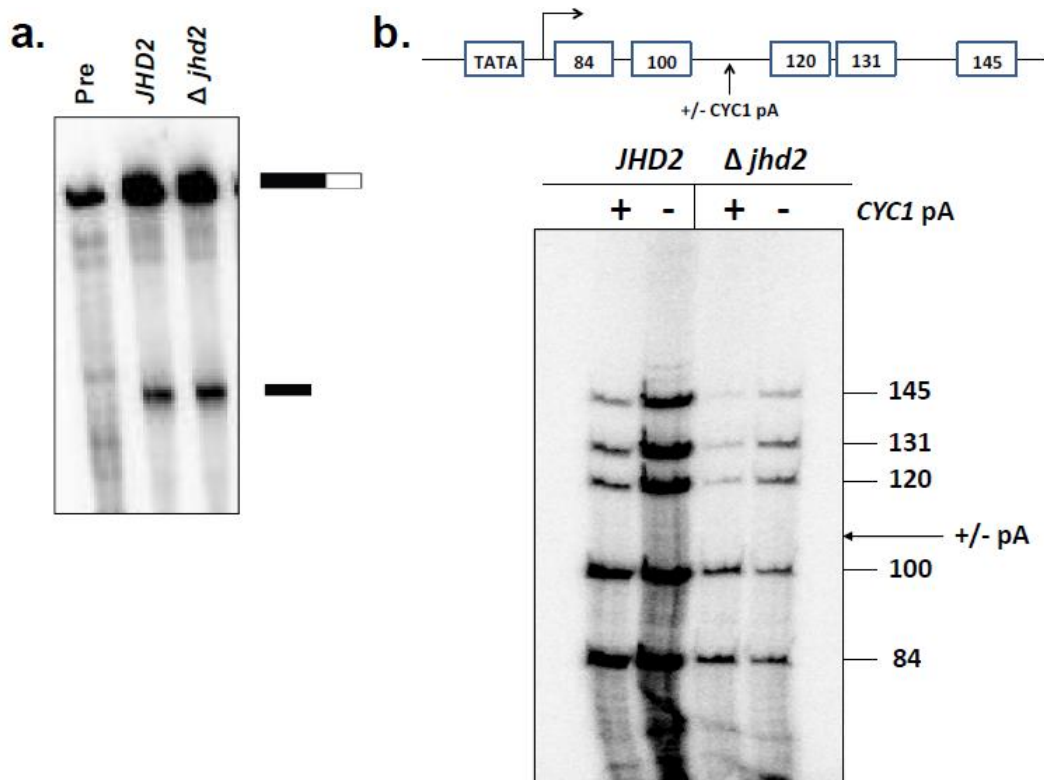
**fig. S1. Mass spectrometry results.** (a) Representation of proteins found in our mass spectrometric analysis of proteins affinity purifying with Jhd2. MS/MS confirmation of Yra1 (b) and Hrp1 (c).



**fig. S2. Western blot analysis of TAP-tagged proteins.** TAP-tagged protein levels of input and co-affinity purifications from (a) TAP-tagged strains overexpressing FLAG-Jhd2 without (left panel) and with (right panel) RNase treatment or (b) Hrp1-TAP or wild-type strains expressing endogenous levels of Jhd2. Membranes were blotted with HRP-conjugated rabbit IgG which recognizes the Protein A portion of the TAP tag. N.D., not determined.



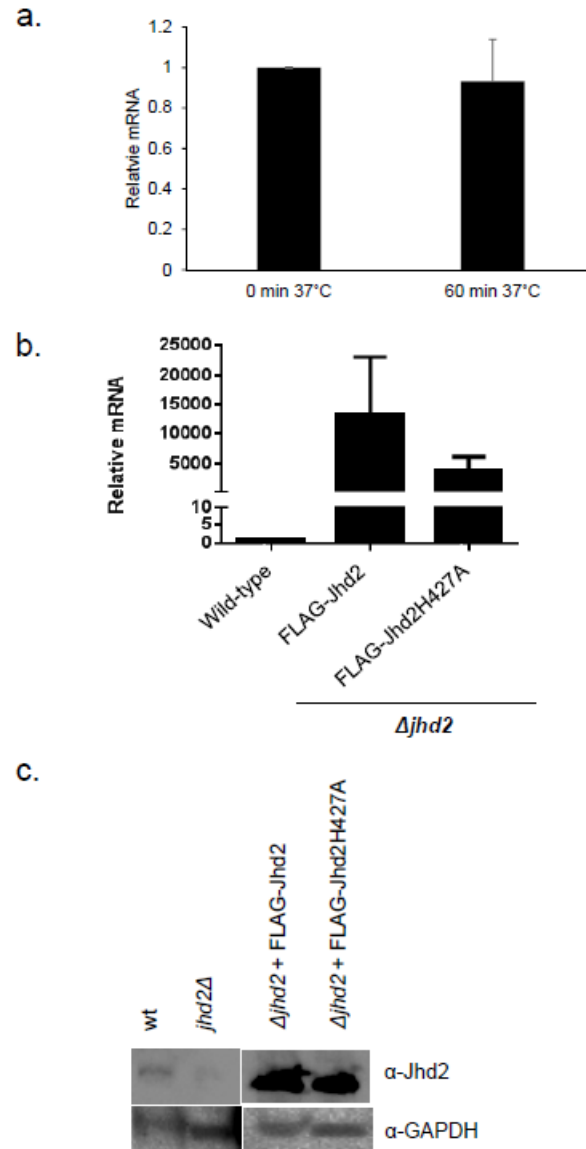
**fig. S3. Materials for in vitro RNA binding experiments.** (a) Verification of recombinant 6xHIS-Jhd2 purification using anti-Jhd2 and anti-HIS antibodies. (b) Sequence of RNA 20-mer used in in vitro RNA binding assays. (c) Three different RNA secondary structure prediction programs show no secondary structure of the random 20-mer. (d) Predicted domain structure of Jhd2 contains a PHD finger, which could bind nucleic acids.



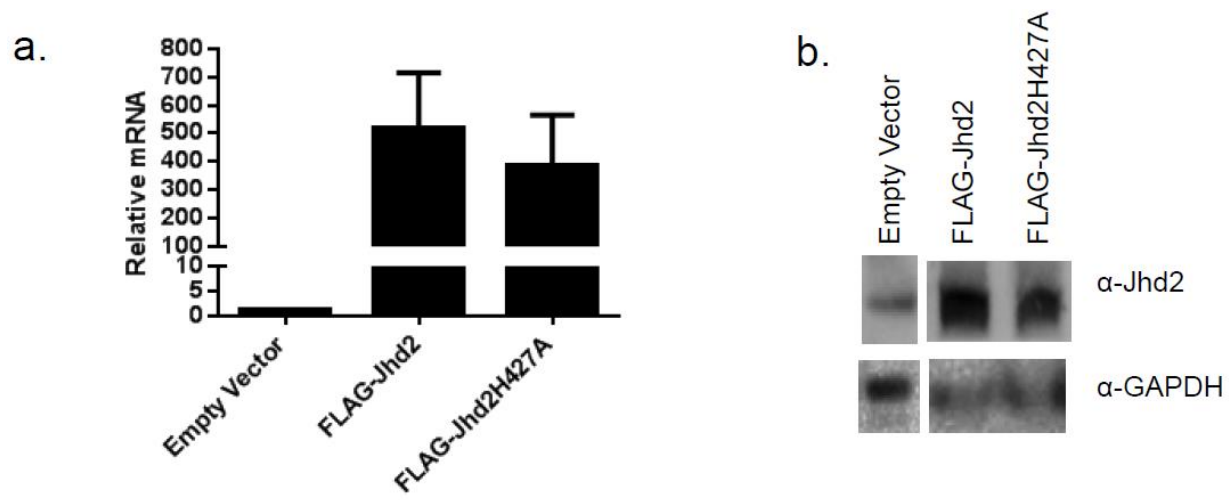
**fig. S4. Loss of Jhd2 does not affect processing or polyA-dependent termination in vitro. (a)**

*In vitro* cleavage reactions with extracts from wild-type cells or *jhd2* deleted cells. Unreacted precursor RNA is shown in the lane marked (Pre), and the positions of precursor and the upstream cleavage product indicated on the right side. The downstream cleavage product is rapidly degraded because it lacks a protective cap structure and is not visible in these reactions.

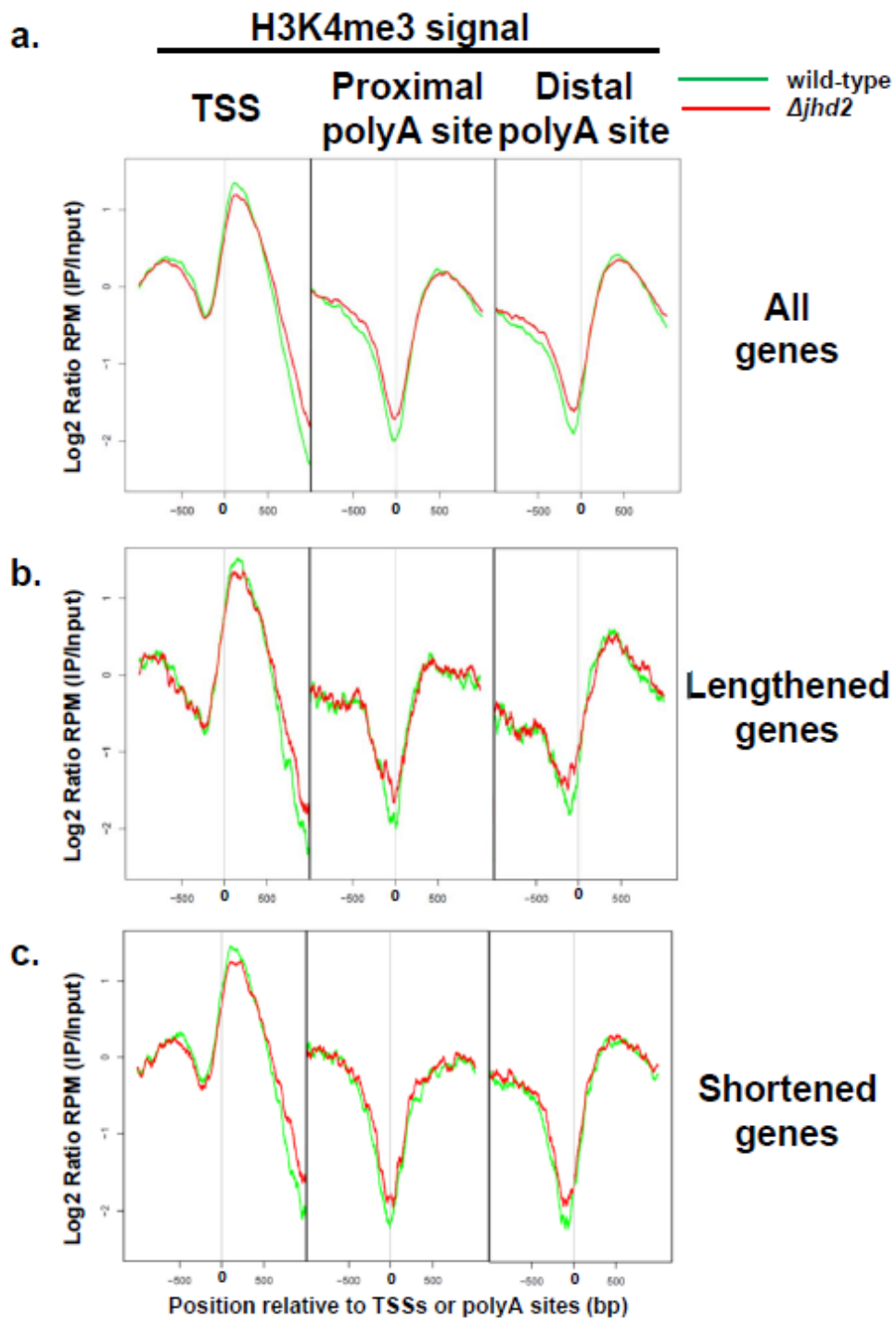
**(b)** *In vitro* transcription reactions with extracts from wild-type cells or *jhd2* deleted cells and the DNA templates diagrammed at the top of the panel. The signal derived from upstream cassettes (84 and 100 nt) is compared to that from the downstream cassettes (120, 131, and 145 nt) to determine the effect of a polyA site. Regardless of whether the extract was made from cells with or without *JHD2*, less RNA was recovered from cassettes located downstream of the polyA site (lanes marked “+”) compared to that from templates lacking the polyA site (lanes marked “-”).



**fig. S5. RT-qPCR and Western blot analysis for Jhd2 mRNA and protein used in *PMA1* studies. Jhd2 mRNA (a, b) and protein (c) levels in the indicated strains.**



**fig. S6. RT-qPCR and Western blot analysis for Jhd2 mRNA and protein used in *KICYC1* studies. mRNA (a) and protein (b) levels of Jhd2 overexpressing strains.**





**fig. S7. Metagene plots of H3K4me3 levels.** Metagene plots of H3K4me3 levels near the TSSs (left), proximal polyA sites (middle), or distal polyA sites (right) of (a) all genes, genes showing (b) lengthened transcripts and (c) shortened transcripts. RPM, reads per million mapped reads.

**table S1. Jhd2 bound peaks identified using SICER.** TSS and TTS denote the start and end of ORFs, respectively. Provided as an excel file.

**table S2. Pcf11 bound peaks identified using SICER.** TSS and TTS denote the start and end of ORFs, respectively. Provided as an excel file.

**table S3. List of genes with APA after Jhd2 deletion.** Fold change represents the distal ratio in Jhd2 deletion stain versus that in wild-type strain. Provided as an excel file.

**table S4. Plasmids used in this study.**

Name	Description	Reference
LPB054	pAD4M	Nat Struct Mol Biol. 2007 Mar; 14(3):243-5.
LPB055	pAD4M-FLAG-JHD2 (WT)	Nat Struct Mol Biol. 2007 Mar; 14(3):243-5.
LPB056	pAD4M-FLAG-JHD2H427A	Nat Struct Mol Biol. 2007 Mar; 14(3):243-5.
LPB057	KICYC1	Yeast 2001; 18:1347-1355
LPB059	pRSF Duet	Novagen
LPB062	JHD2 in LPB059	this study

**table S5. Yeast strains used in this study.** Plasmids introduced to the yeast strains are listed in table S4.

Name	Genotype	Reference
LBY001	MATa JHD2::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Open Biosystems
LBY017	MATa PCF11::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Open Biosystems
LBY024	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	BY4741
LBY025	MATa leu2-3, 112 ura3-52 can1-100 ade2-1 his3-11 trp1-11 bar1Δ	Yeast, 2005; 22(1):43-56
LBY028	MATa ura3-1 trp1D ade2-1 leu2-3,112 his3-11,15 pcf11-2	Mol. Cell. Biol., 1997, 17(3) 1102-1109.
LBY029	MATa jhd2::KAN his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	Open Biosystems
LBY038	MATa his3 Δ 1 leu2Δ0 lys2Δ0 ura3Δ0	BY4742
LBY039	MATa jhd2::KAN his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 [LPB054]	This study
LBY040	MATa jhd2::KAN his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 [LPB055]	This study
LBY041	MATa jhd2::KAN his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 [LPB056]	This study
LBY060	MATa JHD2::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY061	MATa PBP1::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY062	MATa MPE1::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY063	MATa PAP1::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY064	MATa FIP1::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY065	MATa YTH1::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY067	MATa CFT2::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY068	MATa REF2::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY069	MATa RNA14::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY070	MATa PFS2::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY071	MATa RNA15::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY072	MATa PT11::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY073	MATa PCF11::TAP-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [LPB055]	This study
LBY075	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; CLP1::TAP::HIS3MX6	Mol. Cell. Biol., 2012, 32(7) 1321-1331.
LBY076	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; HRP1::TAP::HIS3MX6	Mol. Cell. Biol., 2012, 32(7) 1321-1331.
LBY081	MATa leu2-3, 112 ura3-52 can1-100 ade2-1 his3-11 trp1-11 bar1Δ [LPB054+LPB057]	This study
LBY082	MATa leu2-3, 112 ura3-52 can1-100 ade2-1 his3-11 trp1-11 bar1Δ [LPB055+LPB057]	This study
LBY083	MATa leu2-3, 112 ura3-52 can1-100 ade2-1 his3-11 trp1-11 bar1Δ [LPB056+LPB057]	This study
LBY090	MATa ura3-52 his3Δ200 leu2Δ 1 trp1Δ63 met15Δ0 lys2-128 gal10Δ56	RNA (2007), 13:1756-1764
LBY091	MATa ura3-52 his3Δ200 leu2Δ 1 trp1Δ63 met15Δ0 lys2-128 gal10Δ56 [LPB054]	This study
LBY092	MATa ura3-52 his3Δ200 leu2Δ 1 trp1Δ63 met15Δ0 lys2-128 gal10Δ56 [LPB055]	This study
LBY093	MATa ura3-52 his3Δ200 leu2Δ 1 trp1Δ63 met15Δ0 lys2-128 gal10Δ56 [LPB056]	This study
LBY094	MATa his3D1 leu2Δ0 lys2Δ0 ura3Δ0 [LPB054]	This study

**table S6. Primers used in this study.**

<b>Name</b>	<b>Reference/Comments</b>	<b>Sequence</b>
JHD2 F	this study	TCGCAAGACAAATGATCCAA
JHD2 R	this study	CCGTTTCCGACGATACAAGT
scGAPDH F	this study	TCTTCCATCTTCGATGCTTCCGCT
scGAPDH R	this study	TAAGCCTTGGCAACGTGTTCAACC
PMA1-1 F	Mol Cell, 33, 215-226	TGTATTTCTAATGCGGCACT
PMA1-1 R	Mol Cell, 33, 215-226	CCCgAAAGGCATATGGATAACA
PMA1-2 F	Mol Cell, 33, 215-226	TCGACGACGAAGACAGTGATA
PMA1-2 R	Mol Cell, 33, 215-226	CCGTAAGATGGGTCAGTTTGTAAT
PMA1-4 F	Mol Cell, 33, 215-226	ACTGCTCAAAGTGTAGCGA
PMA1-4 R	Mol Cell, 33, 215-226	TTCCTTAGCAATACCAACAGCATCA
PMA1-5 F	Mol Cell, 33, 215-226	GTCTGGATCTGGTCTATCGG
PMA1-5 R	Mol Cell, 33, 215-226	CGTTCATCAATCTGTCAAAGGC
PMA1-6 F	Mol Cell, 33, 215-226	GCCGCTTATTTCTACTAGAGTT
PMA1-6 R	Mol Cell, 33, 215-226	GGTGTGTGTGTGGATAAAATATTAGAATG
PMA1-7 F	Mol Cell, 33, 215-226	TGCAAGATGTTTGTATAGAGGGAC
PMA1-7 R	Mol Cell, 33, 215-226	ATAGAAGAGCTGGGCAGGA
KICYC-CDS F	this study	AACGTCTGTGGGATGAACA
KICYC-CDS R	this study	TCGTTTCTGTCCTTTTCTTC
KICYC-3UTR F	this study	ACACCGTGAGTTTTGCCTTA
KICYC-3UTR R	this study	TGATCTTTTGATCTTTATGACGAAG
YNL289W 1F	this study, for CDS	ATTCTGCTCAAGTCGCCAGT
YNL289W 1R	this study, for CDS	GCAGAGTGGGTGTGTAGACG
YNL289W 2F	this study, for 3'UTR	TGCAGAAGTTTTCTCTCAGTGG
YNL289W 2R	this study, for 3'UTR	CCGGTTCGCACTACGATACT
YHR103W 1F	this study, for CDS	GTTCCAGCGAGATGCTTCCTA
YHR103W 1R	this study, for CDS	TCCAGCCCTTGACCTTCCTAA
YHR103W 2F	this study, for 3'UTR	GTTTCCCAATTGTTGCTGGT
YHR103W 2R	this study, for 3'UTR	CCATAAAATTCTCCGCATGT
YHR215W 1F	this study, for CDS	GGTGGTTCTGGGCCATACTA

YHR215W 1R	this study, for CDS	AAGACAATGCTCCGCTGAAT
YHR215W 2F	this study, for 3'UTR	TTCCTTGCCTTACTTTTCTTATTATTT
YHR215W 2R	this study, for 3'UTR	AAACAAAATGCGTTTATGACAGTT
hGAPDH F	JBC, 288:9408-9417	CGAGATCCCTCCAAAATCAA
hGAPDH R	JBC, 288:9408-9417	GTCTTCTGGGTGGCAGTGAT
CCND1 1F	this study	AACTACCTGGACCGCTTCCT
CCND1 1R	this study	CCACTTGAGCTTGTTACCA
CCND1 2F	this study	ACGCTTTGTCTGTCGTGATG
CCND1 2R	this study	GTGCAACCAGAAATGCACAG
KDM5B F	JBC, 288:9408-9417	CCATAGCCGAGCAGACTGG
KDM5B R	JBC, 288:9408-9417	GGATACGTGGCGTAAAATGAAGT
KDM5A F	Cell Reports 6, 868–877	CCATAGCCGAGCAGACTGG
KDM5B R	Cell Reports 6, 868–877	GGATACGTGGCGTAAAATGAAGT
DICER1 1F	Nature, 510: 412–416	CTCATTATGACTTGCTATGTCGCCTTG
DICER1 1R	Nature, 510: 412–416	CACAATCTCACATGGCTGAGAAG
DICER1 2F	Nature, 510: 412–416	TGCTTTCCGCAGTCCTAACTATG
DICER1 2R	Nature, 510: 412–416	AATGCCACAGACAAAAATGACC