Supporting online material

HP1 forms distinct complexes to direct histone deacetylation and DNA methylation

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Supplementary Figure 1. Mutant lacking CDP-2 shows aberrant DNA methylation. (**a**) Schematic diagram of Neurospora HP1 and chromodomain proteins, CDP-1, CDP-2, CDP-3 and CDP-4. Numbers indicate amino acid residues. (**b**) Genomic DNA of a wildtype strain (WT), a DNA methyltransferase mutant (*dim-2*) and the *cdp-2* mutant were digested with 5mC-sensitive *Ava*II and *Hpa*II, gel-fractionated and visualized with ethidium bromide. Positions of size standards (kb) are shown at left. (**c**) Global DNA hypermethylation in the *cdp-2* mutant. DNA was digested with 5mC-sensitive *Bfu*CI (B) or its 5mC-insensitive isoschizomer, *Dpn*II (D), gel-fractionated and analyzed by Southern hybridizations with the indicated probes.

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Supplementary Figure 2. DNA methylation of wildtype (WT) and *cdp-2* strains. (a) DNA methylation profile determined by MeDIP. Methylated DNA was immunoprecipitated (MeDIP) from WT and *cdp-2* strains and used to probe approximately 40,000 oligonucleotide sequences on an Agilent slide⁵. Data are shown as log₂[IP per input] values (*y*-axis). Blue and Red indicate RIP product and substrate index, respectively. The indicated 47 peaks of enrichment were previously described⁵. The scale bar on the top left indicates 100 kb. (b) Expanded representation of methylated regions of chromosome VII (scale bar indicating 2 kb is shown at the top left, above peak 1). The positions of predicted genes are shown in gray below the MeDIP data. (c) Methylation patterns of five representative regions in chromosome VII identified by the MeDIP analysis in the *cdp-2* mutant were verified by Southern blotting. The positions of predicted genes are shown at left. (d) Hypermethylation of centromere DNA in *cdp-2* mutant. DNA methylation was analyzed by Southern hybridizations with the indicated probes.

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Supplementary Figure 3. CDP-2 stability depends on HP1 but not *vice versa* and the global level of H3K9me3 does not change in a *cdp-2* mutant. (**a**) Extracts from strains expressing HP1-GFP in WT and *cdp-2* strains were analyzed by Western blotting with antibodies against GFP. (**b**) Extracts from a strain that does not express CDP-2-Flag (-) and strains expressing CDP-2-Flag in WT and *hpo* strains were analyzed by Western blotting with antibodies against Flag. An arrow indicates CDP-2-Flag and asterisks indicate non-specific bands. (**c**) Histones extracted from the indicated strains were subjected to Western blotting using antibodies against H3K9me3 or H3K4me2.

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Supplementary Figure 4. Distribution of DNA methylation, H3K9me3 and HP1 on chromosome VII. (a) Enrichment with anti-5mC antibodies and anti-H3K9me antibodies in WT and *cdp-2* strains, and enrichment with anti-HP1-GFP in WT strains are shown as $log_2[IP \text{ per input}]$ values (*y*-axis). The indicated 47 peaks of enrichment were as described⁵. The scale bar on

the top left indicates 100 kb. (**b**) Expanded representation of methylated regions of chromosome VII. The positions of predicted genes are shown in gray at the bottom. Data in all panels are shown at the same magnification and a scale bar indicating 2 kb is shown at the top left (peak 1).

a

CDP-2

Mass: 62314 pl: 6.80 Coverage: 35.31%

MAGKPVSGRP KTTIEIPLPS IKKYTRGSGP PPPPITLAPP RDSTAYIDQ FVLPVLKDTK PDSRRRIFYH IGFTDIPAAR LLVPCDEVLD YVSPRELEEW EYDALQIKEA DKAVEAEQRR KNQEQAPAKK KPGRPPKARL NEPALDAPAE PVISLQEGDV LLAKQEAVAG PSLATPQKRK RAEIPRFDDS DEAAIHLQLQ NQSPVPSEVE DSGTDLEGYD STDPLSADLG PKTTSYSRAN SSGSSAQQPV AGPSKPSATN PSVSSNLASR PSSSVVSTFG RIHPMFARSI ETGRISVSGH GGQKGAGQKG AGQNGVGHSR GFGGLNAEAI ARASPSRDNS TRQFKAVPKA AVLPFTHPTQ IAPISFTPLA APIAVSRPIV TSDSLSKQVE SSSARKRRKD EQPKPSRKRK KSHIEEPTDS WVVKELLDDQ WVTEHGVKVH KYLVLWEGNW PPDQNPTWEP EDNIDDQGLI KTYLKKKESG MLKAPKKTQR SMLSYLSQPQ YSSVAEAFEG DIDELQPEAA AVTPESDSDD GDELLVTEEP VAKTQKNGNG EKSSFTSFD SKLEQYQNTF PR

HP1

Mass: 30300 pl: 4.74 Coverage: 25.94%

MPYDPSALSD EEAASSVELD TRSATSSSKK QSRDKKSVKY TIPEPEDFED EEQNGDGADE GGEDDEEGDE EEEDVYVVEK **ILDHMLNDDN EPLFLVK**WEG YEK<mark>KSDQTWE PEDTLIEGAS ER</mark>LKEYFTKI GGREKIFEAS AAAQKIKKRG RPSSNSGTPQ ASSNKRSRKN GDHPLNSEEP QTAKNAAWKP PAGSWEEHIA QLDACEDEDT HKLMVYLTWK NGHKTQHTTD VIYKRCPQKM LQFYERHVRI IKRDPDSEDR EGSVS

CHAP

Mass: 62709 pl: 5.51 Coverage: 21.92%

MPYDPDLYPD DDPIDNFNYD PKDDYDELGD DYDPDLDPNQ QGDHEEDVDE FYDAEDVEDE PSLQAPVKVP QHPRRATLSP LQPSRSERHA TPTSTSVRDG TPRSARVAVM LPVSVKKEAY ISVPDVASDE EEEEEEKEKE EDTMVGSFVT SDLMPPTPKR RKITERPSTK PTPTVPAPNY VPPKVQPPPF PIPDIPAPVP LVNPTKKRGR PFGWRPGLSY AAMRGNPVPP PRFVPKQFK APSEVKRGR PPKKPHELPR EIFSKLTPRY IRFLCEWEGC PAELHNFETL RKHVLVVHGD YRQPHQHHLL SAREQPQEPK TCKWASCHSK RLQSELPPLT LPTRHFEAH VNESHLIPFL WHVGDGPRNT SIESPLSEKP LTITSALPSQ PLSSSISHL DVTTTTTNT TTTSTAIKLQ PLPPVLFTS GNQVTFSVRD QLVENDDDKR RRRVRLERVH FLRDENAAPE PVYTQAERDA MEASLAAKKK KQDEFWEYYE KVMGPVVEVT VLAAQQEDLE VPENASGNLS KEVKRKMLSC GWDPQWRGLY QD

HDA-1

Mass: 83710 pl: 5.31 Coverage: 18.28%

NVDNDNDIVM DQQDTHPATI DVSATSNGIT KTEPNGHDQS APANDEKEEK LDDEKDPFIY PARLKRRGLL PTGCCYDDRM KLHANADFGP NPHHPEDPSR IEHLMRIFKK EGUVFTGDDE DLKKVIRTPP RRYMWRIPAR HATKEEICIV HHPEHFRWVE DLSRKPTSEL RRLSTIMDQG RDSLYVGSMT FEAALISAGG ALETCKSVVV GNVKNAFAVI RPPGHHAEFD APMGFCLFNN VPIAAKICQT FYPEICRKIL ILDWDVHHGN GIQNMFYDDP NILYISLHVV DNPMTPDGSI ENCGAGPGLG KNVNIGWHDQ GMCDGETMAA FQXIVMPIAH EFDPDLVIIS AGFDAADGDE LGACFVSPAC YHHTHMLMS LANGKVAVCL EGGYNLAAIS KSALAVARTL MGEPPFKMDL PKINKEAARV LAKVQAYQAP YWECMRPGIV DVQEMQSQGG QRLHDVVRSA QRFNLSEKFG MFPLYIQRDV LFKSFENQVL VTRNASEARK LLVVIHDPE LHAQPDPLDN SLQPHDSWIT DSIPQYIQMA INRKIGVIDI NIPQVITHPE DTESLAPKVD ERTQQAQIQE LMCYIWDNYL QLVDNVDDIF LMGVGNAYLG VKLLLINRDV KSRISGVVNF VNGNLRPVKS DVDTDLSSWY KDNSRVYVSA DHACWADADL SKVMKRRFG SVIRSNVSGL GRMINEHFKD VQDMWVDRWV PEEEKLGDEM VEDA

b

	coverage
DIM-2-FLAG	49.2%
HP1	36.1%
Histone H2A	34.3%
Histone H2B	24.8%
Histone H3	10.3%
Histone H4	49.5%

Supplementary Figure 5. Identification of HCHC components by mass spectrometry. (a) Excised bands shown in Figure 5a were digested with trypsin and peptides analyzed by tandem mass spectrometry. The names of the identified proteins, their molecular masses, the pls and the sequence coverages (percentage of protein sequence detected) are shown. Peptides detected by tandem mass spectrometry are indicated in red.
(b) Mass spectrometry analysis of proteins affinity purified with DIM-2-Flag. The names of the identified proteins and sequence coverages (percentage of protein sequence detected) are shown.



Supplementary Figure 6. Interaction of DIM-2 with HP1 does not depend on CDP-2 and comparison of HP1, DIM-2 and CDP-2 expression levels. (**a**-**b**) HDA-1 and CHAP associate with HP1 *in vivo*. Extracts from strains with (+) or without (-) the HA-tagged *hda-1* or *chap* gene and/or the Flag-tagged *hpo* gene were immunoprecipitated with anti-HA antibodies. Input and IP samples were fractionated and analyzed by Western blotting with the indicated antibodies. (**c**) Extracts from wildtype and *cdp-2* strains expressing DIM-2-Flag and HP1-GFP were subjected to immunoprecipitation with anti-Flag and anti-GFP antibodies (**d**) Whole extracts from strains that express HP1-Flag, DIM-2-Flag or CDP-2-Flag from their native promoters were fractionated and analyzed by Western blotting with anti-Flag antibodies. Asterisks indicate non-specific bands.



Supplementary Figure 7. Effects of *cdp-2*, *hda-1* and *chap* mutants on the distribution of DNA methylation and on accessibility to DIM-5 and DIM-2. (**a-c**) Mutants lacking HDA-1 and CHAP show hypomethylation at moderately RIP'd regions (red bars) and hypermethylation at centromeres. (**a**) The DNA methylation profile of wildtype (WT), *cdp-2*, *hda-1* and *chap* strains across Neurospora LGVII. (**b**) Data for seven representative methylated regions of LGVII (positions indicated by arrows) are shown expanded ~50-fold. (**c**) Confirmation of aberrant DNA methylation in *Cap 2*, *hda-1* and *chap* mutants by Southern blotting.



(d) HCHC is required for DIM-5 recruitment to the moderately RIP'd regions. DamID was performed with the indicated strains. Digested DNA was used for Southern hybridizations with probes for a hypomethylated, moderately RIP'd region (peak 34), a less affected region (8:G3), and the euchromatic gene, *pan-1*. (e) Defects in HCHC complex cause increased accessibility of DIM-2 to centromere region. DamID was performed with the indicated strains. Southern blots were probed with the slightly hypomethylated 8:G3 region, the hypermethylated, heavily RIP'd cenIVR region, and the euchromatic gene, *pan-1*.





Supplementary Figure 8. Function of HCHC in histone acetylation and centromere silencing and its relation to DIM-2. (a-d) Conventional ChIP analyses with antibodies against H3K9me3 and H3K4ac, K9ac, K14ac and K64ac in the indicated strains at two moderately RIP'd regions, peak 33b (a) and 8:A6 (b), and two heavily RIP'd regions, cenVIIR (c) and peak 33a (d). Data from duplicate ChIP experiments were averaged and are presented graphically with error bars to indicate s.d. (e) Silencing defects of reporter gene inserted at centromere VII (CenVIIM::bar^m) in HCHC mutants were examined by serial dilution analyses on nonselective (control) and basta media. (f and g) Synthetic growth defects of *dim-2* and HCHC mutants. (f) Linear growth rates of three wild-type, *dim-2*, *chap* double mutants. Nature Structural & Molecular Biology: doi:10.1038/nsmb.2274

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Strain	Genotype	Reference
N150	mat A	FGSC#2489
N623	mat A his-3	FGSC#6103
N1877	mat a his-3; Δdim -2:: hph^+	Kouzminova & Selker
		(2001)
N2264	mat a his-3; dim-5 leu-2 pan-1	Tamaru & Selker (2001)
N2556	mat a his-3; hpo ^{RIP2}	Freitag et al (2004)
N2700	mat a his-3 cdp -1 ^{$RIP1$}	this study
N2930	mat A his-3 Δ mus-52::bar ⁺	Honda & Selker (2008)
N3018	mat a ;trp-2; Δ mus-52::hph ⁺	this study
N3135	mat a $\triangle cdp-2::hph^+$	FGSC #11771
N3318	mat a his-3; dim-5-3xHA::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3319	mat a his-3; hpo-3xFLAG::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3320	mat A his-3; hpo-3xFLAG::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3321	mat a his-3; hpo-gfp::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3322	mat A his-3: hpo-gfp::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3323	mat a his-3: dim-2-3xFLAG::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3324	mat A his-3: dim-2-3xFLAG::loxP::hph ⁺ ::loxP	Honda & Selker (2008)
N3343	mat a his 3^+ ··· P_{adv} ··· cdn-2-3xFLAG \wedge cdn-2·· hnh ⁺	this study
N3365	mat a his-3 hda-1-3xFLAG··loxP··hnh ⁺ ··loxP	this study
N3366	mat A his-3. hdg-1-3xFLAG. loxP. hnh ⁺ . loxP	this study
N3367	mat A his-3: hda-1-3xHA:·loxP··hnh ⁺ ··loxP	this study
N3368	mat a his-3: hda-1-3rHA:·lorP··hnh ⁺ ··lorP	this study
N3375	mat a his-3; han 3 stilloxphtoxi	this study
N3376	mat 4 his-3; chap-3xFLAG:.tox1nphtox1	this study
N3377	mat A his-3, chap- $3xHA\cdots$ loxP::hph ⁺ :·loxP	this study
N2278	mat A his 2: chap $3xHA$: $lox P$: hph^+ : $lox P$	this study
N3378	mat a his 2; hno $2xEI AC::loxD::hnh^+::loxD: chan = 2xHA::loxD::hnh^+::loxD$	this study
N3575	mat 4 his 2: A hda 1: hnh ⁺	ECSC #12002
N2610	mat A his-3, $\Delta haa-1hph$	FGSC #12003
N2612	mat $u, \Delta naa-1npn$	FOSC #12003
N3012	mat $a, \Delta chap \dots hph^+$	FGSC #12802
N3015	mat A , $\Delta chap: hph$	ruse #12803
N3015	mai A nis-5 \triangle cap-2::npn	this study
N3704	mat a $nis-3$; $npo-3xFLAG::loxP::npn:::loxP; nad-1-3xHA::loxP::npn:::loxP$	this study
N3705	mat a his-3; hpo- $3xFLAG$::loxP::hph ::loxP; hda-1 - $3xHA$::loxP::hph ::loxP	this study
N3752	mat a his-3 :: P_{cdp-2} :: cdp-2-3xFLAG \triangle cdp-2:: hph	this study
N3759	mat a his-3 Δcdp -2::hph; hpo-gfp::loxP::hph ::loxP	this study
N3760	mat $A \Delta cdp$ -2::hph ⁺ ; hpo-gfp::loxP::hph ⁺ ::loxP	this study
N3766	mat $A \Delta cdp$ -2::hph	this study
N3767	mat a his-3 $\triangle cdp$ -2::hph	this study
N3790	mat a his-3 ⁺ :: P_{ccg-1} ::cdp-2-gfp Δcdp -2::hph	this study
N3791	mat A his-3':: P_{ccg-1} ::cdp-2-gfp $\triangle cdp$ -2::hph'	this study
N3804	mat a his-3 \triangle cdp-2::hph'; hpo-3xFLAG::loxP::hph'::loxP;	this study
	chap -3xHA::loxP::hph ::loxP	
N3805	mat A his-3; hpo-3xFLAG::loxP::hph ⁺ ::loxP;	this study
	<i>chap -3xHA::loxP::hph</i> ⁺ :: <i>loxP</i>	
N3808	mat A his- 3^+ :: P_{cdp-2} :: cdp-2-3xFLAG \triangle cdp-2:: hph ⁺ ;	this study
	hda-1-3xHA::loxP::hph ⁺ ::loxP	
N3809	mat a his- 3^+ :: P_{cdp-2} ::cdp-2-3xFLAG \triangle cdp-2::hph ⁺ ;	this study
	hda-1-3xHA::loxP::hph ⁺ ::loxP	
N3864	his-3 ⁺ ::P _{dim-5} ::dim-5-dam	Lewis et al (2010)
N3865	$his-3^+::P_{dim-5}::dim-5-dam; \Delta dim-7::hph^+$	Lewis et al (2010)
N3907	mat a; Δcdp -3:: hph^+	FGSC #11772

Supplementary Table I. Neurospora crassa strains used in this study

N3908	mat A: Δcdp -3:: hph^+	FGSC #11773
N3909	mat a: Δcdp -4:: hph^+	FGSC #12173
N3910	mat A: Δcdp -4:: hph^+	FGSC #12174
N3911	$his-3^+::P_{cca,1}::cdp-2-gfp \Delta cdp-2::hph^+: trp-2: dim-5::bar$	this study
N3912	$his-3^+::P_{acg}:::cdp-2-gfp \wedge cdp-2::hph^+: trp-2: dim-2^{RIP32}$	this study
N3913	$his-3^+::P_{ccg_1}::cdp-2-gfp \Delta cdp-2::hph^+: trp-2: hpo^{RIP2} trp-2$	this study
N3914	$+his-3^+::P_{acc}:::cdp-2-rfp: nan-2::P_{acc}:::hpo-gfp$	this study
N4005	mat $A \Delta cdp - 2$:: hph^+ his- 3^+ :: P_{dim-5} :: $dim-5$ -dam	this study
N4018	mat a his- 3^+ :: P_{dim} 5::dim-5-dam: hpo ^{RIP2}	this study
N4019	mat A his- 3^+ :: P_{dim-5} ::dim-5-dam; $\Delta dim-2$::hph ⁺	this study
N4020	mat a his-3 ⁺ :: P_{dim-5} ::dim-5-dam: $\Delta dim-2$::hph ⁺	this study
N4048	mat a his-3; dim-2-dam:: $loxP::hph^+::loxP$	this study
N4049	mat A his-3: dim-2-dam::loxP::hph ⁺ ::loxP	this study
N4092	mat $A \wedge cdn-2$:: hph^+ his-3: $dim-2$ - dam :: $loxP$:: hph^+ :: $loxP$	this study
N4093	mat $A \Delta cdp - 2$:: hph^+ his-3: $dim - 2$ - dam :: $loxP$:: hph^+ :: $loxP$	this study
N4094	mat a his-3: dim-2-dam::loxP::hph ⁺ ::loxP: hpo ^{$RIP2$}	this study
N4095	mat A his-3: dim-2-dam:: $loxP::hph^+::loxP: hpo^{RIP2}$	this study
N4676	mat a his-3: dim-2-dam::loxP::hph ⁺ ::loxP: Δ hda-1::hph ⁺	this study
N4677	mat A his-3: dim-2-dam::loxP::hph ⁺ ::loxP: Δ hda-1::hph ⁺	this study
N4678	mat A: dim-2-dam::loxP::hph ⁺ ::loxP: Δ chap::hph ⁺	this study
N4679	mat a his-3: dim-2-dam::loxP::hph ⁺ ::loxP: Δ chap::hph ⁺	this study
N4696	mat a his-3 $\triangle cdp$ -2:: hph^+ ; $\triangle dim$ -2:: hph^+	this study
N4714	mat A: $\Delta chap::hph^+$: $\Delta dim-2::hph^+$	this study
N4715	mat a; $\Delta chap::hph^+$; $\Delta dim-2::hph^+$	this study
N4890	mat A: CenVIR::bar ^m ; trp-2; his-3	this study
N4891	mat a; CenVIR::bar ^m hpo; his-3	this study
N4892	mat a; CenVIIM:: bar^{m} ; $trp-2$	this study
N4893	mat a; CenVIIM:: bar^m	this study
N4894	mat a; CenVIIM:: bar^m ; $trp-2$; hpo	this study
N4895	mat a; CenVIIM:: bar^{m} ; his-3 Δcdp -2:: hph^{+}	this study
N4896	mat A; CenVIIM:: bar^{m} ; his-3 Δcdp -2:: hph^{+}	this study
N4897	mat a; CenVIIM:: $bar^m \Delta dim - 2$:: hph^+ ; his-3	this study
N4903	mat A; CenVIR::bar ^m ; trp-2; his- 3 ; Δ hda-1::hph ⁺	this study
N4905	mat a; CenVIR:: bar^{m} ; trp-2; his-3; Δdim -2:: hph^{+}	this study
N4906	mat a; CenVIR::bar ^m ; trp-2; his-3; $\Delta h da - 1$:hph ⁺	this study
N4907	mat A; CenVIR::bar ^m ; trp-2; his-3; $\Delta dim-2$::hph ⁺	this study
N4909	mat a; CenVIR::bar ^m ; trp-2; his-3	this study
N4911	mat A; CenVIR::bar ^m ; trp-2; his-3; Δ chap::hph ⁺	this study
N4912	mat a; CenVIR::bar ^m ; trp-2; his-3; $\Delta chap$::hph ⁺	this study
N4915	mat A; CenVIR::bar ^m ; trp-2; Δcdp -2::hph ⁺	this study
N4916	mat a; CenVIR:: bar^m ; trp-2; Δcdp -2:: hph^+	this study
N4941	mat a; CenVIIM:: bar ^m ; his-3; $\Delta h da - 1$:: hph ⁺	this study
N4942	mat A; CenVIIM::bar ^m ; his-3; Δhda -1:: hph^+	this study
N4943	mat A: CenVIIM:: bar^{m} : $\Delta chap::hph^{+}$	this study

Primer	Sequence						
his-3 targetting vectors							
cdp-2-GFP							
#1276	5'- GCCACTAGTATGGCCGGCAAGCCTGTCAGCGGC-3'						
#1277	5'- GCCGGATCCCCCTTGGGAATGTCTTCTGGTACT-3'						
cdp-2-RFP							
#1276	5'- GCCACTAGTATGGCCGGCAAGCCTGTCAGCGGC-3'						
#3152	5'- CUCAGATUTTCACUTTGGGAATGTUTT-3'						
cdp-2-HA							
#3151	5'- CCGGCGGCCGCAAAGGTGTTCCAGTCCAGCAC-3'						
#3157	5'- GCCTTAATTAACCCTTGGGAATGTCTTCTGGTACT-3'						
Dam construct							
	5'_Ο Ο ΤΤ Δ Δ ΤΤ Δ Δ G Δ Δ Δ Δ Δ Τ Ο G Ο G Ο ΤΤΤΤΤΤ G Δ_3'						
#3150	5'-TCCCCCCGGGTCATTTTTTCGCGGGTGAAA-3'						
115150	5 Teeeeeeoooreximmedeoooronimit 5						
MBP-CDP-2							
#3153	5'-GCCGGCTCCCGTCGAAAAGACGAGCAACCG-3'						
#3154	5'-GCCGTCGACTCACCTTGGGAATGTCTTCTG-3'						
Knock-in							
hda-1 5'							
#2076	5'-GAGGTCGACGGTATCGATAAGCTTGATATGTATATCCAGCGTGACGTGC-3'						
#2077	5'-CCTCCGCCTCCGCCGCCGCCTCCGCCGCGTCCTCCACCATCTCATC-3'						
hda-1 3'							
#2078	5'-TGCTATACGAAGTTATGGATCCGAGCTCGAAACAAGACGGGCCTTGGTC-3'						
#2079	5'-ACCGCGGTGGCGGCCGCTCTAGAACTAGTGGTTCCGGCAAGGATAGAGG-3'						
chap 5'							
#2080	5'-GAGGTCGACGGTATCGATAAGCTTGATATAGACCAGGCTTGTCCTATGC-3'						
#2081	5'-CCTCCGCCTCCGCCGCCGCCTCCGCCATCCTGGTACAACCCCCTCC-3'						
chap 3'							
#2082	5'-TGCTATACGAAGTTATGGATCCGAGCTCGTTTAAGCGGGAGGGCGAAGG-3'						
#2083	5'-ACCGCGGTGGCGGCCGCTCTAGAACTAGTCTCCGGAATTGACATCATGG-3'						
<i>dim-2</i> 5'							
#1988	5'-GAGGTCGACGGTATCGATAAGCTTGATATATCAAGGCCTGGAACAACGG-3'						
#2013	5'-CCTCCGCCTCCGCCGCCCCCCCCCCCCCCCCCCCCCCC						
112013							
<i>dim-2</i> 3'							
#1989	5'-TGCTATACGAAGTTATGGATCCGAGCTCGTGCGACGAGGTAACGCCATG-3'						
#1990	5'-ACCGCGGTGGCCGCCCCTCTAGAACTAGTACAAGACCGGCAACCATCTG-3'						
1770							
Dam vector							
#3058	5'-CCTTAATTAAGGGCGGAGGCGGCGGAGGCGGGGGGGGGG						
	TCGCGCTTT-3'						
#3059	5'-GGAATTCATTTTTCGCGGGTGAAACG-3						

Supplementary Table II. Primers used in this study

Probe Peak11 (949 bp) #2360 #2361	5'-TTGAGCGGTACGTTAGATCC-3' 5'-TCCGGATCCTCTTCCTTAGG-3'
Peak34 (667 bp) #2455 #2456	5'-CGAATACGGCGGATCACGTG-3' 5'-ACCCTGGAGATGGATCTCTG- 3'
cenI LF (864 bp))
#3091	5'-GCCTATACCCGGTTATAGTAAGG-3'
#3092	5'-GCCTTAGTTGTAGGTACTAGG-3'
cenI RF (1106 b	p)
#3093	5'-GTTAGGAGTTTTCATCCAGCG-3'
#3094	5'-GAGATTCTCGGAGTGCAAGG-3'
cenII LF (744 br	D)
#3095	5'-CCGATATTGTAGCGCGTACC-3'
#3096	5'-AGCTAAAACCAGTACGGGTC -3'
cenIII LF (967 b	p)
#3099	5'-CGAACCCTTCCTAGAGATCG-3'
#3100	5'-AGTCCTCGGGGTACTAAAGC-3'
cen III RF (870)	bp)
#3101	5'-AATACGATCCGTCAGAGGCC-3'
#3102	5'-GCTGGATCCCTAGTTCAAGC-3'
cenIV RF (921 b	pp)
#3105	5'-CAAGCCGATAACTCCTAGGC-3'
#3106	5'-GGTATTTTGTCTCCGCCGTTC-3'
cenV LF (994 bj	b)
#3107	5'-TTAGGGGTACAACACCAACC-3'
#3108	5'-GAGGATAGATTGGCAAGAGC-3'
cenVI RF (1158	bp)
#3113	5'-GGAAAAGCCCTACTAGCACC-3'
#3114	5'-CCTCTTCAAGGATGATTTCCTC-3'
cenVII LF (2044	bp)
#2539	5'-CTTCTACTAGACCTAAGGGAGG-3'
#2540	5'-AGAAAAGTCGCGCTCCTAGC-3'
cenVII middle (1	1960 bp)
#2543	5'-TTGGATTCCCTATAGAAGAGAGG-3'
#2544	5'-AATAGCCCTAGAGGCTAGCC-3'
cenVII RF (979	bp)
#2541	5'-GGAGGTATAGAGGTACTAGGAG-3'
#2542	5'-CTCTAGTAAGCTCGATACTCTGG-3'
<i>pan-1</i> (1007 bp) #3181	5'-CGATAAGCTTGATATCGAATTCAGGTTGTCCGGCCATCTCAGTCTGATCC-3'

#3182	5'-TCGCATACGCCAACCCATGC-3'
ChIP	
hH4-1 (425 bp)	
#1924	5'-AACCACCGAAACCGTAGAGGGTAC-3'
#1925	5'-ATCGCCGACACCGTGTGTTGTAAC-3'
8:A6 (303 bp)	
#1823	5'-GGATGGCGGATCCTCAAAAATA-3'
#1824	5'-TAACCGCCGCTTTTTAAAATTAGGA-3'
8:G3 (231 bp)	
#1866	5'-CGLAGAAGGGAAGLAGLAGLAGAAGG-3'
#1867	5-GUAUAATAUGAAGTUAUTTTTUAUU-3
Peak28a (244 h	n)
#2513	5'-CGTTACGGAGCTTTTGATCG-3'
#3028	5'-GGGAGTAACGATCAACCTTCG-3'
10020	
Peak28b (214 bp	
#3029	5'-CCGGACAGGTACATAGGTAC-3'
#2516	5'-AACCTCTGTCAAGGTTGACG-3'
D 100 (0011	х.
Peak33a (221 bp	
#3019	5 - UGAUGUAAUAAAIIAUIAUAUIAIAUAAU- 5
#3020	5-GGIIIICGIAIAGIAAGIIACCCGCIIC-3
Peak33b (251 br	
#3021	'' 5'-AGGTTTTTGTTGTGTGCAGATG-3'
#3022	5'-AGGATGGCTGCAAGGCAGAAC-3'
cenVIIL (261 bp	
#2539	5'-CTTCTACTAGACCTAAGGGAGG-3'
#2550	5'-CGTCTTTGATAGTCGGGGGATAAGG-3'
cenVIIR (294 br	
#2541	" 5'-GGAGGTATAGAGGTACTAGGAG-3'
#2541 #2562	5'-CTTATTAAAGGTGCCCAGATATAATAGTAG-3'
#2302	5-01141144400100000000000000000000000000
Centromere mark	ters
cenIIIL	
#3725	5'-GGTCCCTGTATAAGAAGGTGG-3'
#3726	5'-GCTCCTTCAATATCATCTTCTGTCGACGGCGATCTCTAGGAAGGGTTCG-3'
#3727	5'-AGCTTTGTTCCCTTTAGTGAGGGTTAATTACTTTGAGCTGCCGGTATCC-3'
#3100	5'-AGTCCTCGGGGTACTAAAGC-3'
L // D	
cenVIR	
#3113	5'-GGAAAAGCCCTACTAGCACC-3'
#3728	5-GUIUUTIUAATATUATUTIUTGTUGAUGGUIGAATUTTUGATATUCGAC-3'
#3729 #2720	5 -UTTTOTTCCTTTCCCACAC 22
#3/30	J-ATCTACUCUATCTCUUAUAC-J
cenVIIM	
#2543	5'- TTGGATTCCCTATAGAAGAGAGG -3'
#3733	5'-GCTCCTTCAATATCATCTTCTGTCGACGGAGCGTATTAGCGTACCGAAG'
#3734	5'-GCTTTGTTCCCTTTAGTGAGGGTTAATTACTTCCAAATCGATTGACTGC-3'

#2544 5'-AATAGCCCTAGAGGCTAGCC-3'

Supplemental Methods

Construction of epitope tagged CDP-2 fusion proteins

For the CDP-2-GFP fusion construct expressed by *ccg-1* promoter, the *cdp-2* coding region was amplified by PCR with primers #1276 and #1277 and digested with *SpeI* and *Bam*HI. pMF272¹ was digested with the corresponding restriction enzymes and ligated with the inserts, yielding pTTK5. The N-terminal RFP-CDP-2 construct was similarly generated with primers #1276 and #3152 and pMF334², yielding pTTK28. For HA-tagged CDP-2 expressed by its endogenous promoter, a fragment of the *cdp-2* coding region with its promoter was amplified by PCR with primers #3151 and # 3157 and digested with *NotI* and *PacI*. pMF270³ was digested with *NotI* and *PacI* and ligated with the inserts, yielding pTTK30. Similarly, the Flag-tagged CDP-2 construct was generated with primers #3151 and #1277 and pCCG::C-3xFLAG, yielding pTTK26. All constructs were linearized and inserted into the *his-3* locus of the *cdp-2* null strain by the gene replacement method previously described⁴.

Construction of strains expressing tagged HDA-1 and CHAP fusion proteins from their native loci

Knock-in constructs were generated as previously described for other genes⁴. For example, to generate strains expressing HDA-1-Flag from its native locus, a 1-kb fragment including the 3' end of the *hda-1* coding region, without the stop codon, and a 500-bp fragment of the 3' *hda-1* flanking region were amplified by PCR. The two PCR products were assembled in yeast with linearized pRS416 and a 3xFlag knock-in module. The assembled plasmids were transformed into a $\Delta mus-52$ strain (N2930) by electroporation and a resulting transformant was characterized

and then crossed with $mus-52^+$ strains to recover progeny with the wildtype mus-52 allele. The HDA-1-HA, CHAP-Flag and CHAP-HA knock-in constructs were generated similarly.

Supplemental References

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