Supporting online material

HP1 forms distinct complexes to direct histone deacetylation and DNA methylation

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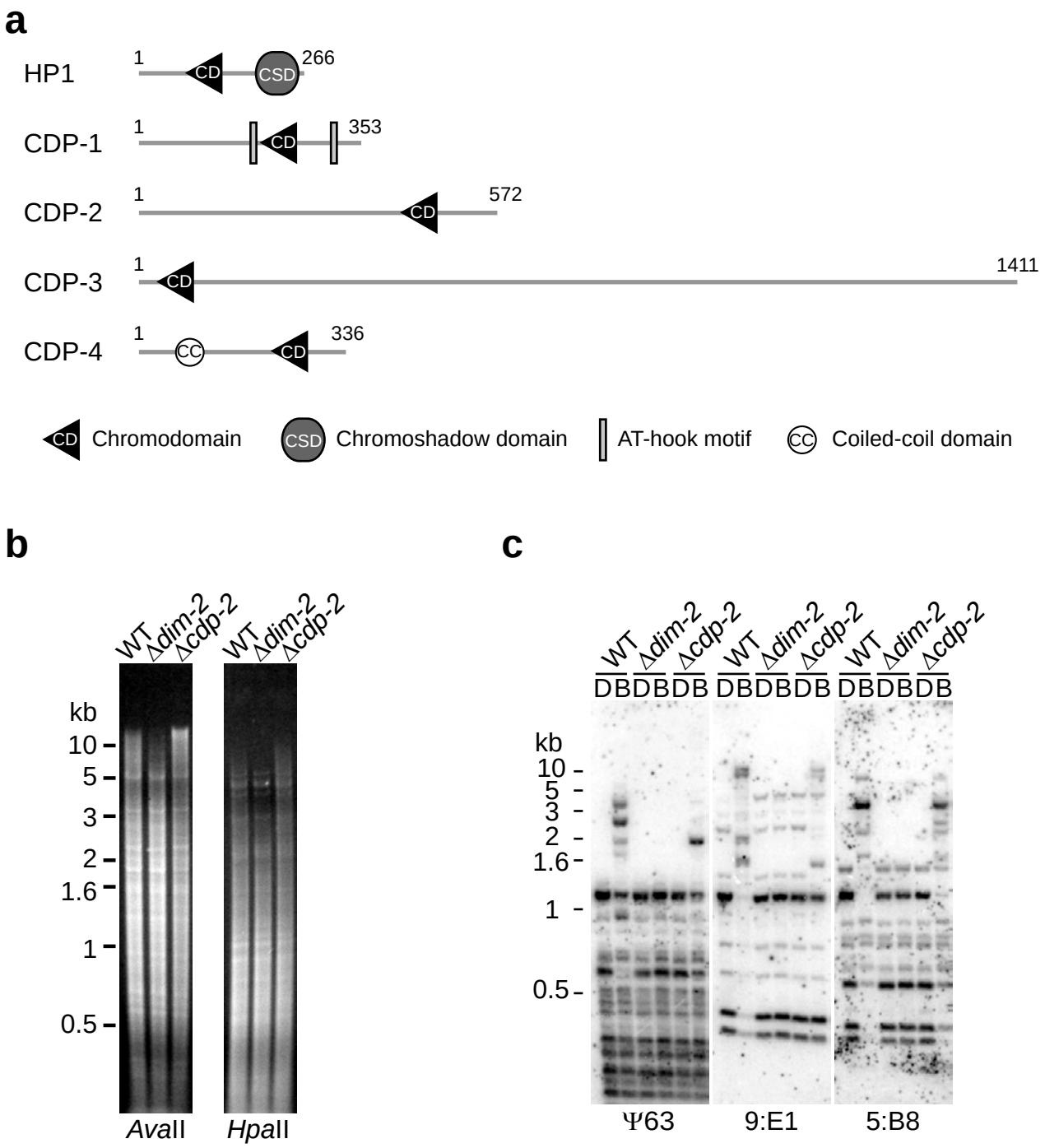
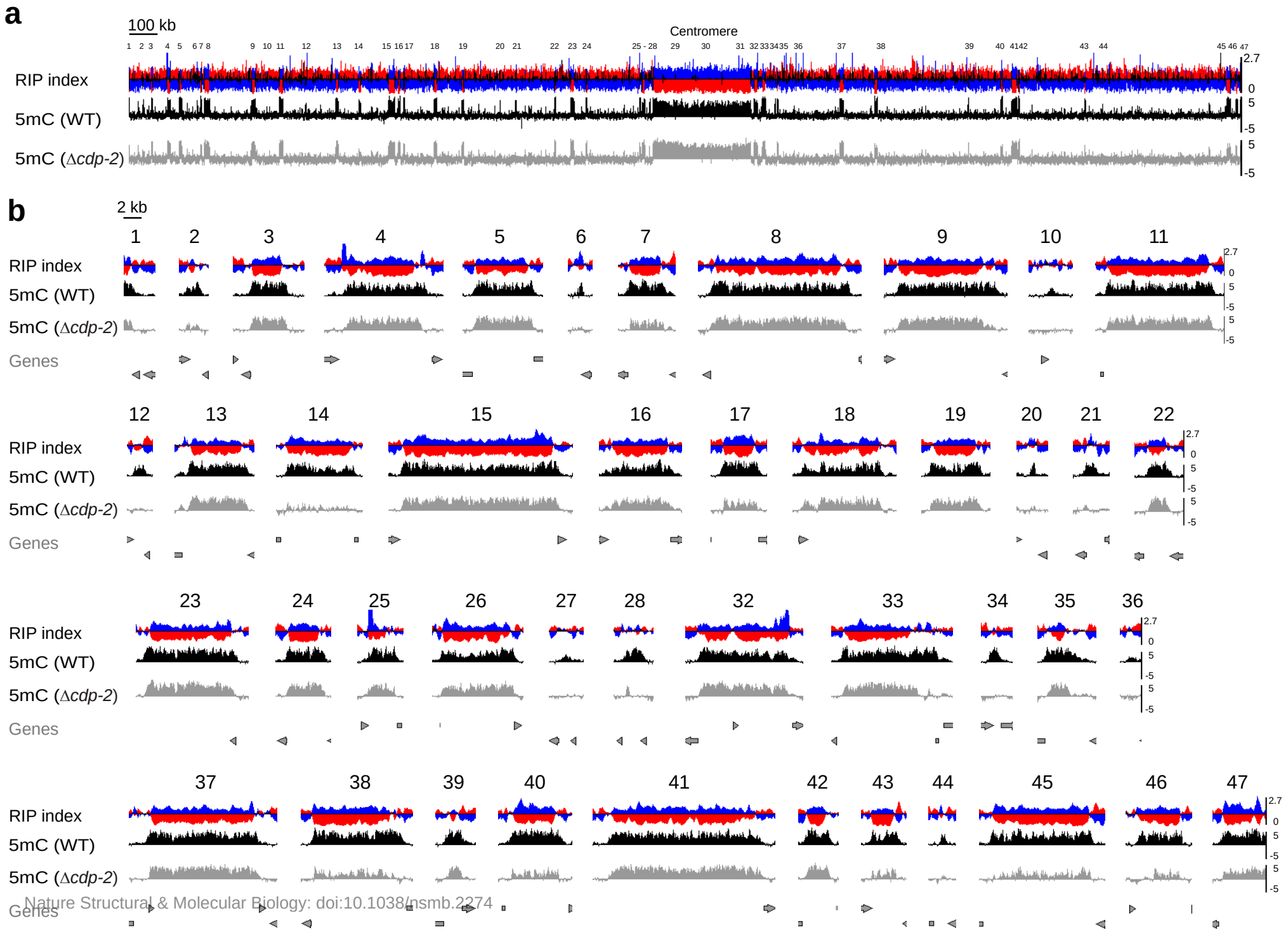


Supplementary Figure 1. Mutant lacking CDP-2 shows aberrant DNA methylation. (**a**) Schematic diagram of Neurospora HP1 and chromodomain proteins, CDP-1, CDP-2, CDP-3 and CDP-4. Numbers indicate amino acid residues. (**b**) Genomic DNA of a wildtype strain (WT), a DNA methyltransferase mutant (*dim-2*) 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.

Honda_Supplementary Fig. 2



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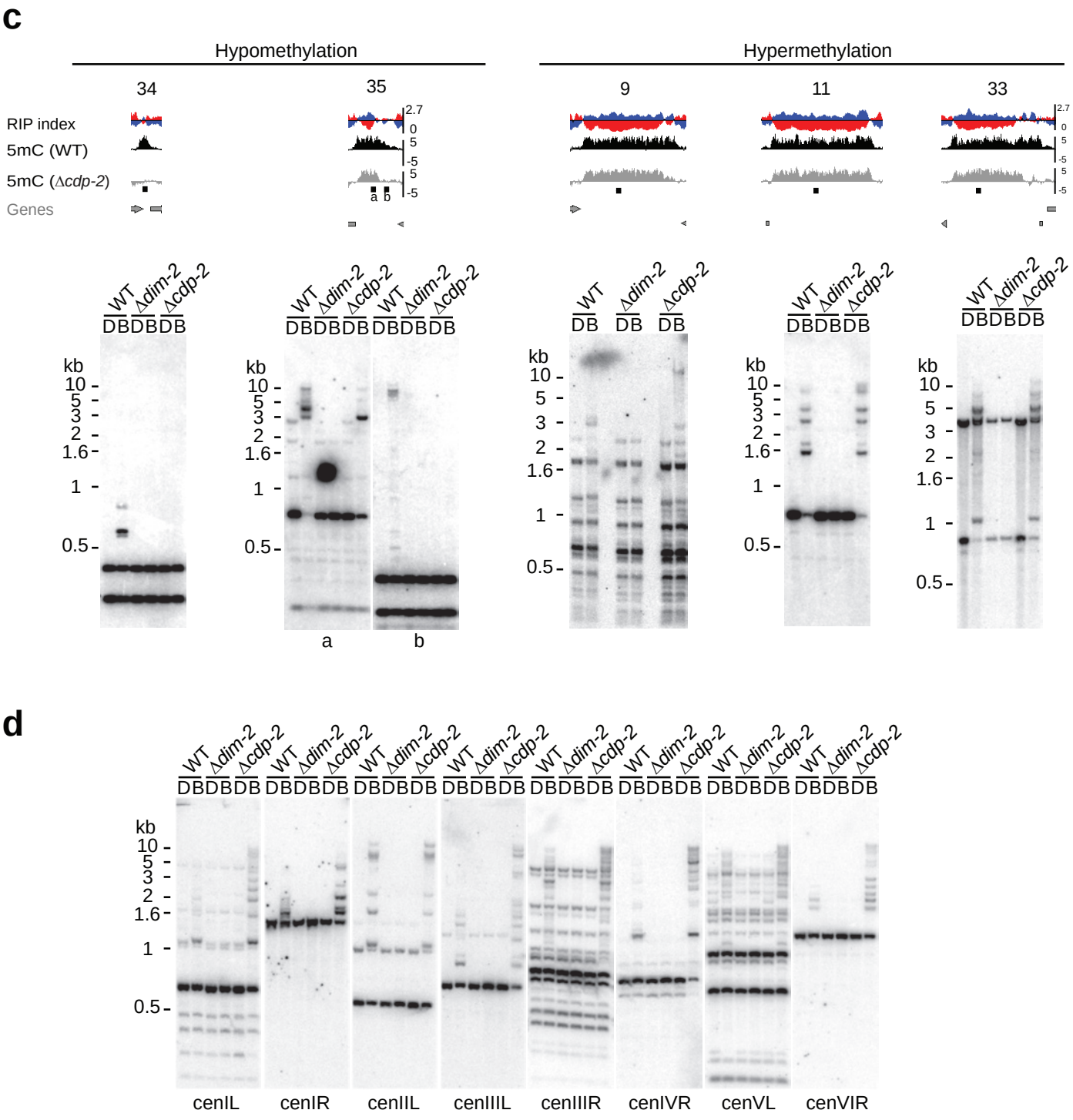

Supplementary Figure 2. DNA methylation of wildtype (WT) and *cdp-2* strains. (**a**) DNA methylation profile determined by MeDIP. Methylated DNA was immunoprecipitated (MeDIP) from WT and *cdp-2* strains and used to probe approximately 40,000 oligonucleotide sequences on an Agilent slide[5]. Data are shown as $\log_2$[IP per input] values (*y*-axis). Blue and Red indicate RIP product and substrate index, respectively. The indicated 47 peaks of enrichment were previously described[5]. 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 in gray at the bottom. The horizontal black bars indicate the sites used for Southern hybridizations. Positions of size standards (kb) 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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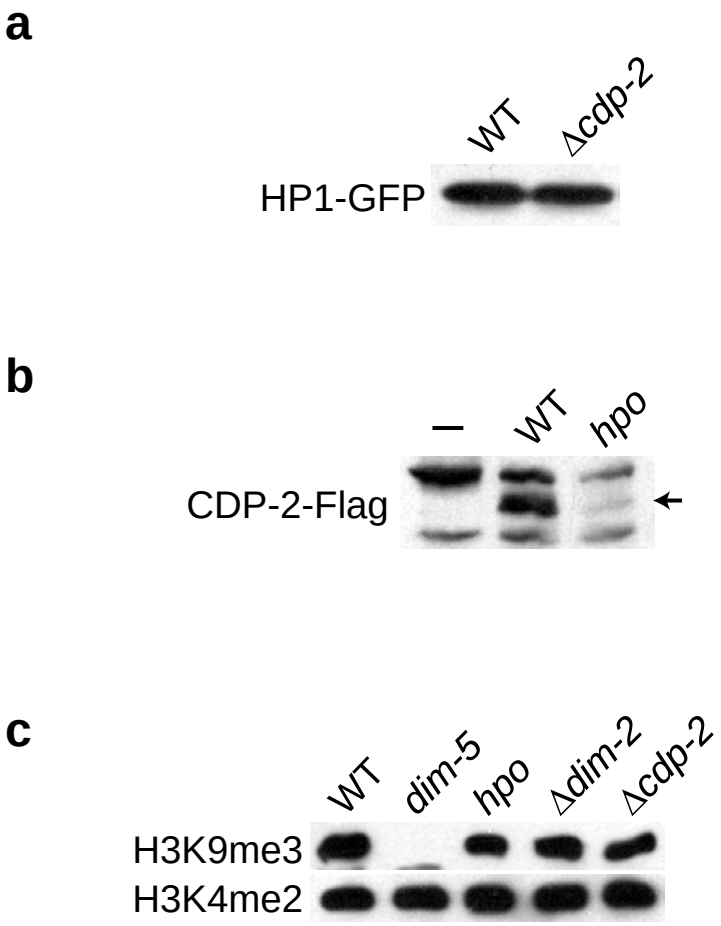
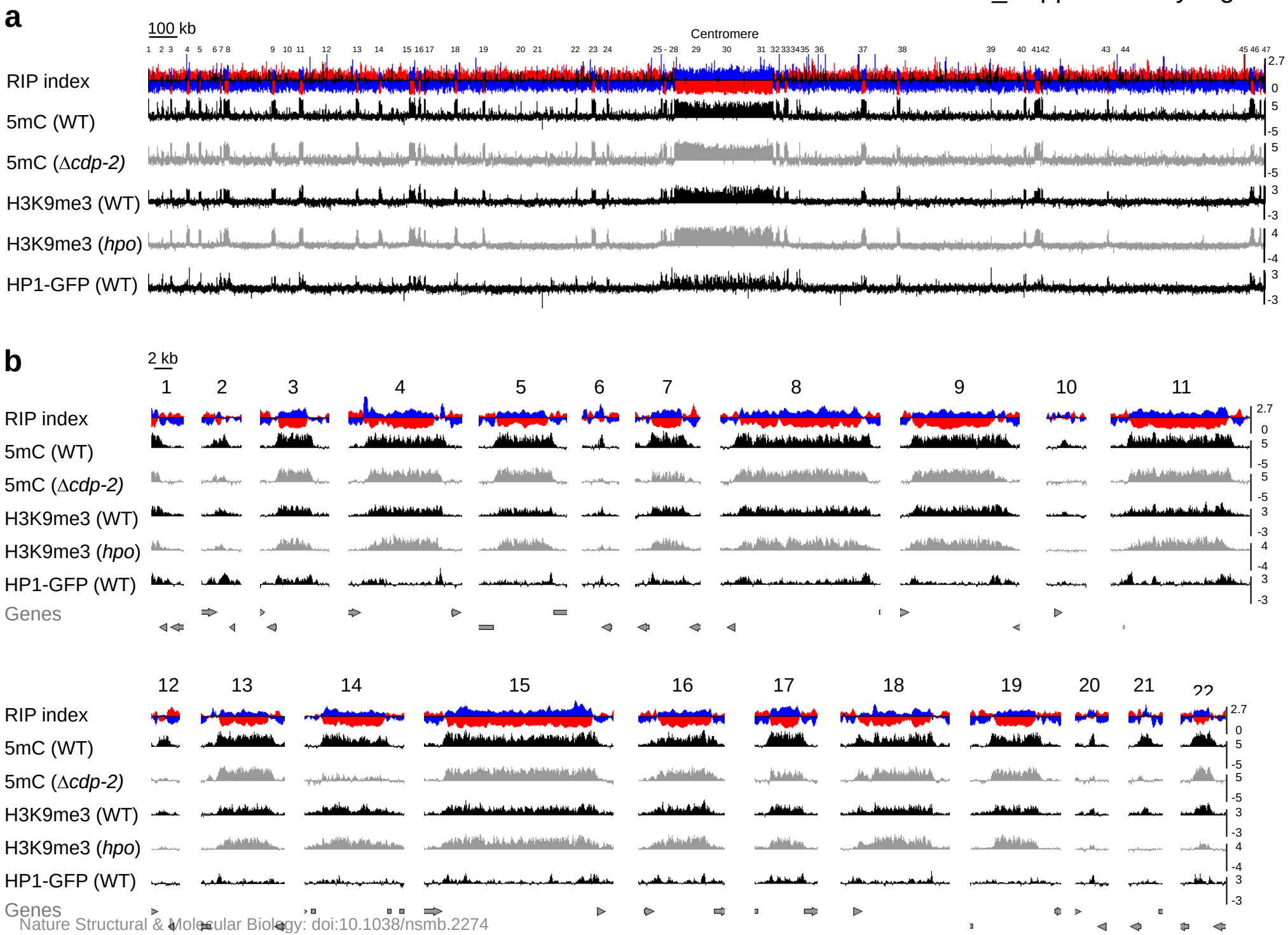


Supplementary Figure 3. CDP-2 stability depends on HP1 but not *vice versa* and the global level of H3K9me3 does not change in a *cdp-2* mutant. (**a**) Extracts from strains expressing HP1-GFP in WT and *cdp-2* strains were analyzed by Western blotting with antibodies against GFP. (**b**) Extracts from 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.

Honda_Supplementary Fig. 4



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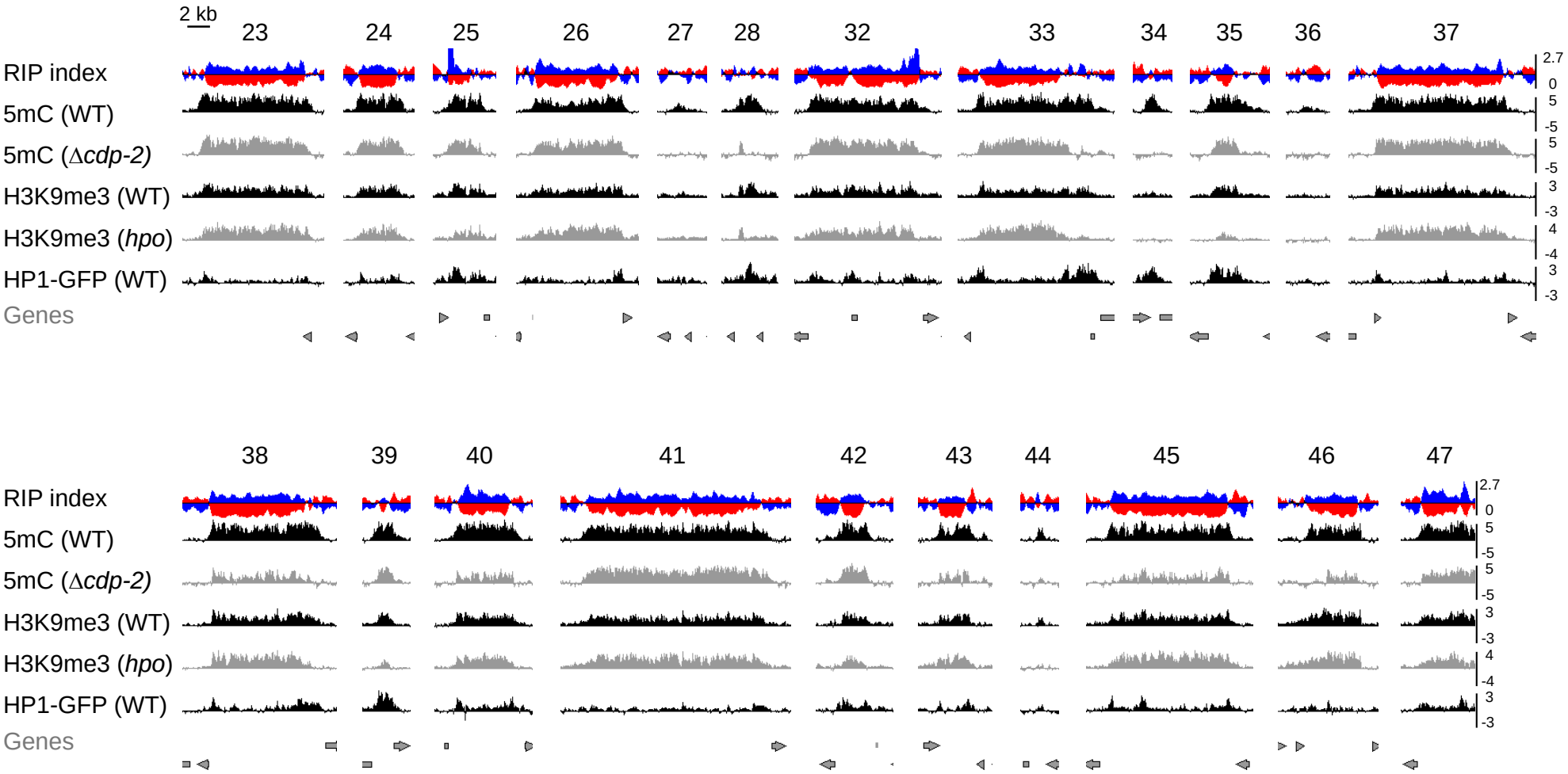

Supplementary Figure 4. Distribution of DNA methylation, H3K9me3 and HP1 on chromosome VII. (**a**) Enrichment with anti-5mC antibodies and anti-H3K9me antibodies in WT and *cdp-2* strains, and enrichment with anti-HP1-GFP in WT strains are shown as $\log_2$[IP per input] values (*y*-axis). The indicated 47 peaks of enrichment were as described[5]. 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 pI: 6.80 Coverage: 35.31%

```
MAGKPVSGRP KTTIEIPLPS IKKYTRGSGP PPPPITLAPP RDSTAYIIDQ FVLPVLKDTK PDSRRRIFYH IGFTDIPAAR LLVPCDEVLD YVSPRELEEW
EYDALQIKEA DKAVEAEQRR KNQEQAPAKK KPGRPPKARL NEPALDAPAE PVISLQEGDV LLAKQEAVAG PSLATPQKRK RAEIPRFDDS DEAAIHLQLQ
NQSPVPSEVE DSGTDLEGYD STDPLSADLG PKTTSYSRAN SSGSSAQQPV AGPSKPSATN PSVSSNLASR PSSSVVSTPG 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 EKSSSFTSFD SKLEQYQKTF PR
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HP1
Mass: 30300 pI: 4.74 Coverage: 25.94%

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MPYDPSALSD EEAASSVELD TRSATSSSKK QSRDKKSVKY TIPEPEDFED EEQNGDGADE GGEDDEEGDE EEEDVYVVEK ILDHMLNDDN EPLFLVKWEG
YEKKSDQTWE PEDTLIEGAS ERLKEYFTKI GGREKIFEAS AAAQKIKKRG RPSSNSGTPQ ASSNKRSRKN GDHPLNSEEP QTAKNAAWKP PAGSWEEHIA
QLDACEDEDT HKLMVYLTWK NGHKTQHTTD VIYKRCPQKM LQFYERHVRI IKRDPDSEDR EGSVS
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CHAP
Mass: 62709 pI: 5.51 Coverage: 21.92%

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MPYDPDLYPD DDPIDNFNYD PKDDYDELGD DYDPDLDPNQ QGDHEEDVDE FYDAEDVEDE PSLQAPVKVP QHPRRATLSP LQPSRSERHA TPTSTSVRDG
TPRSARVAVM LPVSVKKEAY ISVPDVASDE EEEEEEKEKE EDTMVGSFVT SDLMPPTPKR RKITERPSTK PTPTVPAPNY VPPKVQPPPF PIPDIPAPVP
LVNPTKKRGR PFGWRPGLSY AAMRGNPVPP PRPKVPKQPK APSEVKRRGR PPKKPHELPR EIFSKLTPRY IRFLCEWEGC PAELHNFETL RKHVLVVHGD
YRQPHQHHLL SAREQPQEPK TCKWASCHSK RLQSELPPLT LPTRSHFEAH VNESHLIPFL WHVGDGPRNT SIESPLSEKP LTITSALPSQ PLSSSSISHL
DVTTTTTTNT TTTSTAIKLQ PLPPYLFDTS GNQVTPSVRD QLYENDDDKR RRRVRLERVH FLRDENAAPE PVYTQAERDA MEASLAAKKK KQDEFWEYYE
KVMGPVVEVT VLAADQEDLE VPENASGNLS KEVKRKMLSC GWDPQWRGLY QD
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HDA-1
Mass: 83710 pI: 5.31 Coverage: 18.28%

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MVDNDNDIVM DGQDTHPATI DVSATSNGIT KTEPNGHDQS APANDEKEEK LDDEKDPFIY PARLKRRGLL PTGCCYDDRM KLHANADFGP NPHHPEDPSR
IEHIMRIFKK EGLVFTGDDE DLKKVIRTDP RRYMWRIPAR HATKEEICIV HHPEHFRWVE DLSRKPTSEL RRLSTIMDQG RDSLYVGSMT FEAALISAGG
AIETCKSVVV GNVKNAFAVI RPPGHHAEFD APMGFCLFNN VPIAAKICQT EYPEICRKIL ILDWDVHHGN GIQNMFYDDP NILYISLHVY MNGSFYPGKP
DNPMTPDGSI ENCGAGPGLG KNVNIGWHDQ GMGDGEYMAA FQKIVMPIAH EFDPDLVIIS AGFDAADGDE LGACFVSPAC YAHMTHMLMS LANGKVAVCL
EGGYNLAAIS KSALAVARTL MGEPPPKMDL PKINKEAARV LAKVQAYQAP YWECMRPGIV DVQEMQSQGG QRLHDVVRSA QRFNLSEKFG MFPLYIQRDV
LFKSFENQVL VTRNASEARK LLVVIHDPPE LHAQPDPLDN SLQPHDSWIT DSIPQYIQWA INRKIGVIDI NIPQYITHPE DTESLAPKVD ERTQQAQIQE
LMCYIWDNYL QLYDNVDDIF LMGVGNAYLG VKLLLINRDV KSRISGVVNF VNGNLRPVKS DVDTDLSSWY KDNSRVYVSA DHACWADADL SRKVMKRRFG
SVIRSNVSGL GRMMNEHFKD VQDWMVDRMV PEEEKLGDEM VEDA
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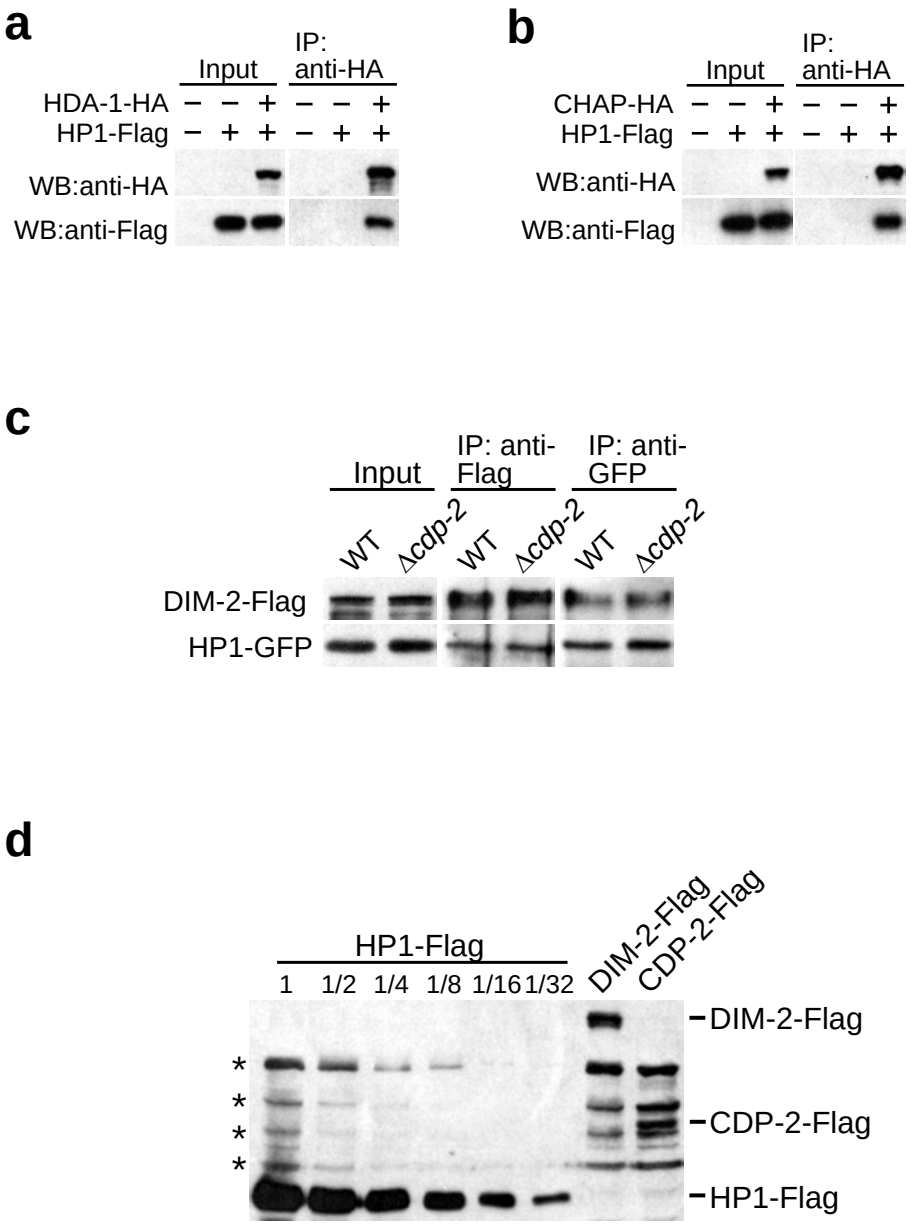
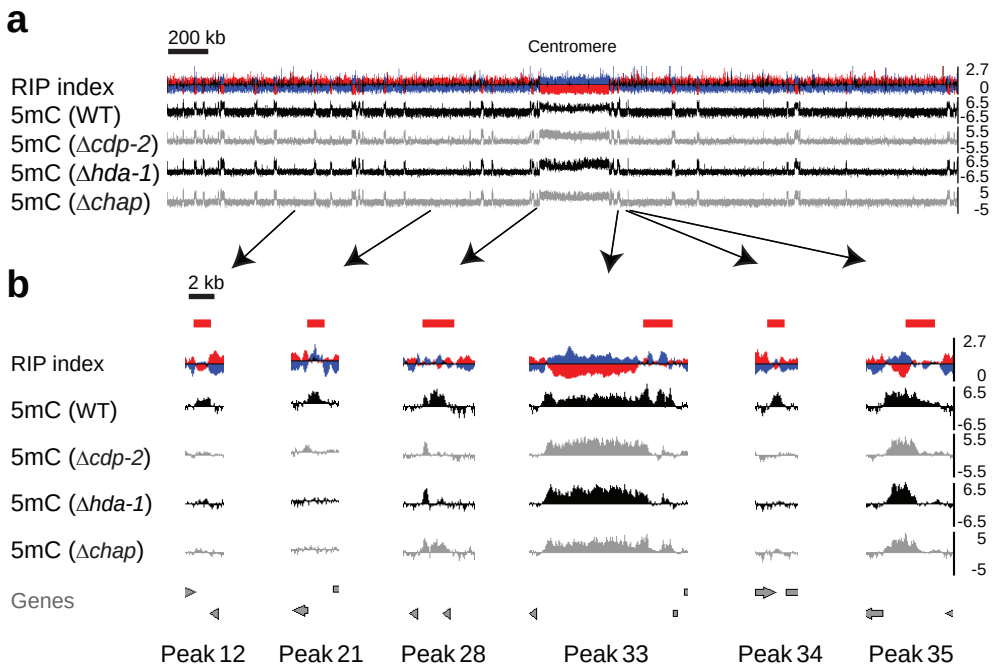
b

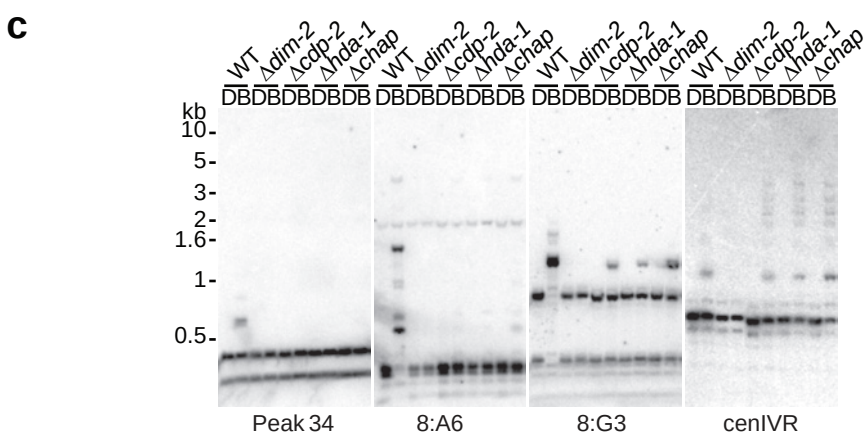
	coverage
DIM-2-FLAG	49.2%
HP1	36.1%
Histone H2A	34.3%
Histone H2B	24.8%
Histone H3	10.3%
Histone H4	49.5%

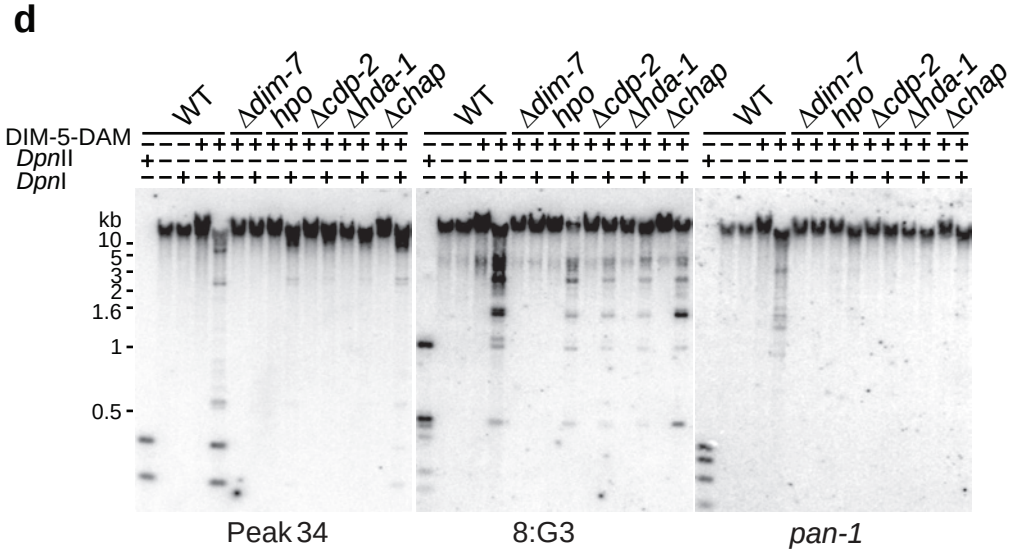
Supplementary Figure 5. Identification of HCHC components by mass spectrometry. (**a**) Excised bands shown in **Figure 5a** were digested with trypsin and peptides analyzed by tandem mass spectrometry. The names of the identified proteins, their molecular masses, the pIs and the sequence coverages (percentage 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.

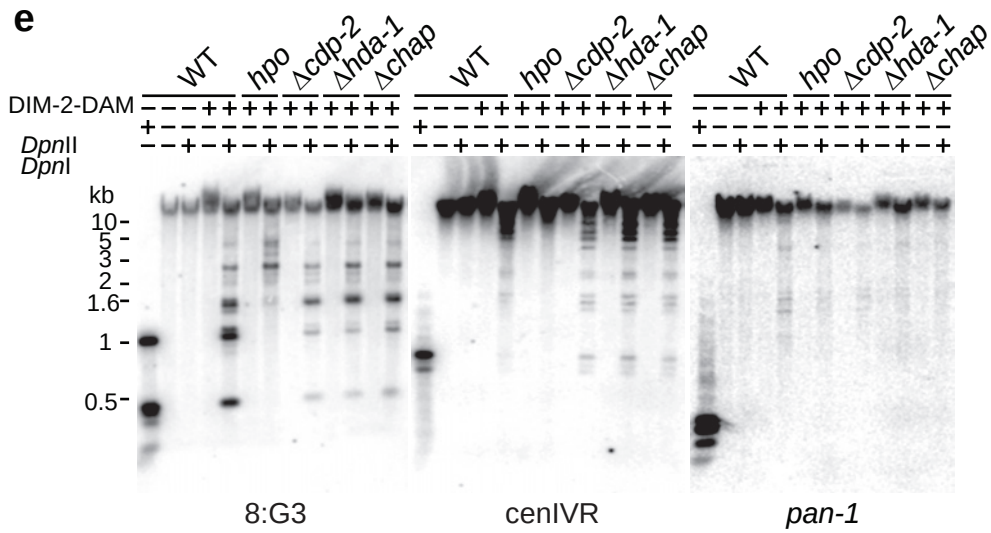


Supplementary Figure 6. Interaction of DIM-2 with HP1 does not depend on CDP-2 and comparison of HP1, DIM-2 and CDP-2 expression levels. (**a**-**b**) HDA-1 and CHAP associate with HP1 *in vivo*. Extracts from strains with (+) or without (-) the HA-tagged *hda-1* or *chap* gene and/or the Flag-tagged *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 *cdp-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*.

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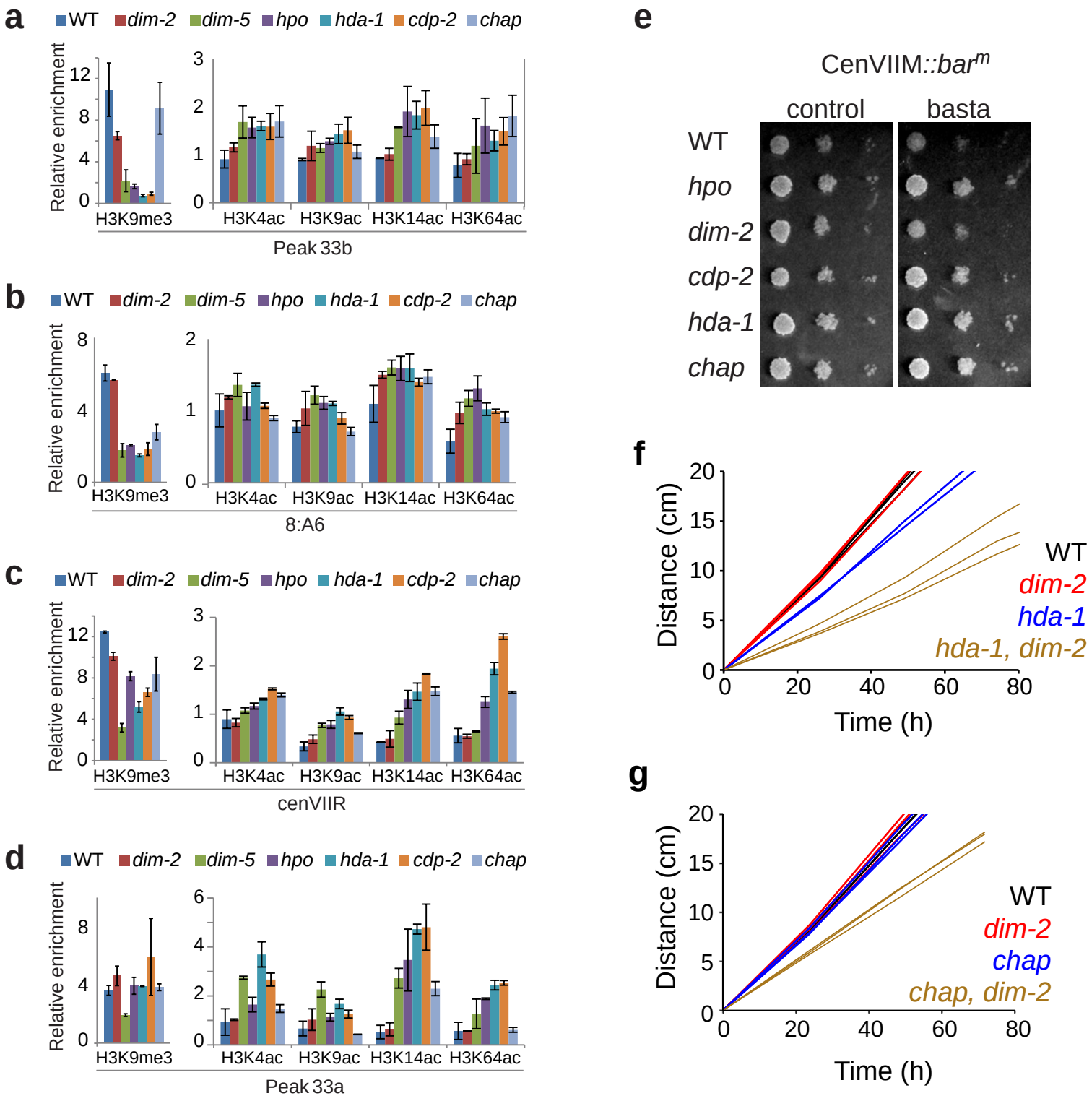

Supplementary Figure 8. Function of HCHC in histone acetylation and centromere silencing and its relation to DIM-2. (**a-d**) Conventional ChIP analyses with antibodies against H3K9me3 and H3K4ac, K9ac, K14ac and K64ac in the indicated strains at two moderately RIP'd regions, peak 33b (**a**) and 8:A6 (**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*, *hda-1*, and *dim-2*, *hda-1* double mutants. (**g**) Linear growth rates of three wild-type, *dim-2*, *chap*, and *dim-2*, *chap* double mutants.

Supplementary Table I. *Neurospora crassa* strains used in this study

Strain	Genotype	Reference
N150	*mat A*	FGSC#2489
N623	*mat A his-3*	FGSC#6103
N1877	*mat a his-3*; Δ*dim-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* Δ*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*$_{cdp-2}$*::cdp-2-3xFLAG* Δ*cdp-2::hph*$^{+}$	this study
N3365	*mat a his-3*; *hda-1-3xFLAG::loxP::hph*$^{+}$*::loxP*	this study
N3366	*mat A his-3*; *hda-1-3xFLAG::loxP::hph*$^{+}$*::loxP*	this study
N3367	*mat A his-3*; *hda-1-3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3368	*mat a his-3*; *hda-1-3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3375	*mat a his-3*; *chap-3xFLAG::loxP::hph*$^{+}$*::loxP*	this study
N3376	*mat A his-3*; *chap-3xFLAG::loxP::hph*$^{+}$*::loxP*	this study
N3377	*mat A his-3*; *chap -3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3378	*mat a his-3*; *chap -3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3393	*mat a his-3*; *hpo-3xFLAG::loxP::hph*$^{+}$*::loxP*; *chap -3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3610	*mat A his-3*; Δ*hda-1::hph*$^{+}$	FGSC #12003
N3610	*mat a*; Δ*hda-1::hph*$^{+}$	FGSC #12003
N3612	*mat a*; Δ*chap::hph*$^{+}$	FGSC #12802
N3613	*mat A*; Δ*chap::hph*$^{+}$	FGSC #12803
N3615	*mat A his-3* Δ*cdp-2::hph*$^{+}$	this study
N3704	*mat a his-3*; *hpo-3xFLAG::loxP::hph*$^{+}$*::loxP*; *hda-1 -3xHA::loxP::hph*$^{+}$*::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* Δ*cdp-2::hph*$^{+}$	this study
N3759	*mat a his-3* Δ*cdp-2::hph*$^{+}$; *hpo-gfp::loxP::hph*$^{+}$*::loxP*	this study
N3760	*mat A* Δ*cdp-2::hph*$^{+}$; *hpo-gfp::loxP::hph*$^{+}$*::loxP*	this study
N3766	*mat A* Δ*cdp-2::hph*$^{+}$	this study
N3767	*mat a his-3* Δ*cdp-2::hph*$^{+}$	this study
N3790	*mat a his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-gfp* Δ*cdp-2::hph*$^{+}$	this study
N3791	*mat A his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-gfp* Δ*cdp-2::hph*$^{+}$	this study
N3804	*mat a his-3* Δ*cdp-2::hph*$^{+}$; *hpo-3xFLAG::loxP::hph*$^{+}$*::loxP*; *chap -3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3805	*mat A his-3*; *hpo-3xFLAG::loxP::hph*$^{+}$*::loxP*; *chap -3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3808	*mat A his-3*$^{+}$*::P*$_{cdp-2}$*::cdp-2-3xFLAG* Δ*cdp-2::hph*$^{+}$; *hda-1-3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3809	*mat a his-3*$^{+}$*::P*$_{cdp-2}$*::cdp-2-3xFLAG* Δ*cdp-2::hph*$^{+}$; *hda-1-3xHA::loxP::hph*$^{+}$*::loxP*	this study
N3864	*his-3*$^{+}$*::P*$_{dim-5}$*::dim-5-dam*	Lewis *et al* (2010)
N3865	*his-3*$^{+}$*::P*$_{dim-5}$*::dim-5-dam*; Δ*dim-7::hph*$^{+}$	Lewis *et al* (2010)
N3907	*mat a*; Δ*cdp-3::hph*$^{+}$	FGSC #11772

N3908	*mat A*; Δ*cdp-3::hph*$^{+}$	FGSC #11773
N3909	*mat a*; Δ*cdp-4::hph*$^{+}$	FGSC #12173
N3910	*mat A*; Δ*cdp-4::hph*$^{+}$	FGSC #12174
N3911	*his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-gfp* Δ*cdp-2::hph*$^{+}$; *trp-2*; *dim-5::bar*	this study
N3912	*his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-gfp* Δ*cdp-2::hph*$^{+}$; *trp-2*; *dim-2*^{RIP32}	this study
N3913	*his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-gfp* Δ*cdp-2::hph*$^{+}$; *trp-2*; *hpo*^{RIP2} *trp-2*	this study
N3914	*+his-3*$^{+}$*::P*$_{ccg-1}$*::cdp-2-rfp*; *pan-2::P*$_{ccg-1}$*::hpo-gfp*	this study
N4005	*mat A* Δ*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*; Δ*dim-2::hph*$^{+}$	this study
N4020	*mat a his-3*$^{+}$*::P*$_{dim-5}$*::dim-5-dam*; Δ*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* Δ*cdp-2::hph*$^{+}$ *his-3*; *dim-2-dam::loxP::hph*$^{+}$*::loxP*	this study
N4093	*mat A* Δ*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* Δ*cdp-2::hph*$^{+}$; Δ*dim-2::hph*$^{+}$	this study
N4714	*mat A*; Δ*chap::hph*$^{+}$; Δ*dim-2::hph*$^{+}$	this study
N4715	*mat a*; Δ*chap::hph*$^{+}$; Δ*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* Δ*cdp-2::hph*$^{+}$	this study
N4896	*mat A*; CenVIIM*::bar*^{m}; *his-3* Δ*cdp-2::hph*$^{+}$	this study
N4897	*mat a*; CenVIIM*::bar*^{m} Δ*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*; Δ*dim-2::hph*$^{+}$	this study
N4906	*mat a*; CenVIR*::bar*^{m}; *trp-2*; *his-3*; Δ*hda-1::hph*$^{+}$	this study
N4907	*mat A*; CenVIR*::bar*^{m}; *trp-2*; *his-3*; Δ*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*; Δ*chap::hph*$^{+}$	this study
N4915	*mat A*; CenVIR*::bar*^{m}; *trp-2*; Δ*cdp-2::hph*$^{+}$	this study
N4916	*mat a*; CenVIR*::bar*^{m}; *trp-2*; Δ*cdp-2::hph*$^{+}$	this study
N4941	*mat a*; CenVIIM*::bar*^{m}; *his-3*; Δ*hda-1::hph*$^{+}$	this study
N4942	*mat A*; CenVIIM*::bar*^{m}; *his-3*; Δ*hda-1::hph*$^{+}$	this study
N4943	*mat A*; CenVIIM*::bar*^{m}; Δ*chap::hph*$^{+}$	this study

Supplementary Table II. Primers used in 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'- CCCAGATCTTCACCTTGGGAATGTCTT-3'
cdp-2-HA	
#3151	5'- CCGGCGGCCGCAAAGGTGTTCCAGTCCAGCAC-3'
#3157	5'- GCCTTAATTAACCCTTGGGAATGTCTTCTGGTACT-3'
Dam construct	
#3149	5'-CCTTAATTAAGAAAAATCGCGCTTTTTTGA-3'
#3150	5'-TCCCCCCGGGTCATTTTTTCGCGGGTGAAA-3'
MBP-CDP-2	
#3153	5'-GCCGGCTCCCGTCGAAAAGACGAGCAACCG-3'
#3154	5'-GCCGTCGACTCACCTTGGGAATGTCTTCTG-3'
Knock-in	
hda-1 5'	
#2076	5'-GAGGTCGACGGTATCGATAAGCTTGATATGTATATCCAGCGTGACGTGC-3'
#2077	5'-CCTCCGCCTCCGCCTCCGCCGCCTCCGCCCGCGTCCTCCACCATCTCATC-3'
hda-1 3'	
#2078	5'-TGCTATACGAAGTTATGGATCCGAGCTCGAAACAAGACGGGCCTTGGTC-3'
#2079	5'-ACCGCGGTGGCGGCCGCTCTAGAACTAGTGGTTCCGGCAAGGATAGAGG-3'
chap 5'	
#2080	5'-GAGGTCGACGGTATCGATAAGCTTGATATAGACCAGGCTTGTCCTATGC-3'
#2081	5'-CCTCCGCCTCCGCCTCCGCCGCCTCCGCCATCCTGGTACAACCCCCTCC-3'
chap 3'	
#2082	5'-TGCTATACGAAGTTATGGATCCGAGCTCGTTTAAGCGGGAGGGCGAAGG-3'
#2083	5'-ACCGCGGTGGCGGCCGCTCTAGAACTAGTCTCCGGAATTGACATCATGG-3'
dim-2 5'	
#1988	5'-GAGGTCGACGGTATCGATAAGCTTGATATATCAAGGCCTGGAACAACGG-3'
#2013	5'-CCTCCGCCTCCGCCTCCGCCGCCTCCGCCCAACTTGACAATCGTCATGC-3'
dim-2 3'	
#1989	5'-TGCTATACGAAGTTATGGATCCGAGCTCGTGCGACGAGGTAACGCCATG-3'
#1990	5'-ACCGCGGTGGCGGCCGCTCTAGAACTAGTACAAGACCGGCAACCATCTG-3'
Dam vector	
#3058	5'-CCTTAATTAAGGGCGGAGGCGGCGGAGGCGGCGGAGGCATGAAGAAAAA TCGCGCTTT-3'
#3059	5'-GGAATTCATTTTTTCGCGGGTGAAACG-3

Probe

Peak11 (949 bp)
#2360 5'-TTGAGCGGTACGTTAGATCC-3'
#2361 5'-TCCGGATCCTCTTCCTTAGG-3'

Peak34 (667 bp)
#2455 5'-CGAATACGGCGGATCACGTG-3'
#2456 5'-ACCCTGGAGATGGATCTCTG- 3'

cenI LF (864 bp)
#3091 5'-GCCTATACCCGGTTATAGTAAGG-3'
#3092 5'-GCCTTAGTTGTAGGTACTAGG-3'

cenI RF (1106 bp)
#3093 5'-GTTAGGAGTTTTCATCCAGCG-3'
#3094 5'-GAGATTCTCGGAGTGCAAGG-3'

cenII LF (744 bp)
#3095 5'-CCGATATTGTAGCGCGTACC-3'
#3096 5'-AGCTAAAACCAGTACGGGTC -3'

cenIII LF (967 bp)
#3099 5'-CGAACCCTTCCTAGAGATCG-3'
#3100 5'-AGTCCTCGGGGTACTAAAGC-3'

cen III RF (870 bp)
#3101 5'-AATACGATCCGTCAGAGGCC-3'
#3102 5'-GCTGGATCCCTAGTTCAAGC-3'

cenIV RF (921 bp)
#3105 5'-CAAGCCGATAACTCCTAGGC-3'
#3106 5'-GGTATTTTGTCTCCGCCGTTC-3'

cenV LF (994 bp)
#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 (1960 bp)
#2543 5'-TTGGATTCCCTATAGAAGAGAGG-3'
#2544 5'-AATAGCCCTAGAGGCTAGCC-3'

cenVII RF (979 bp)
#2541 5'-GGAGGTATAGAGGTACTAGGAG-3'
#2542 5'-CTCTAGTAAGCTCGATACTCTGG-3'

pan-1 (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'-CGTAGAGAAGGGAAGTAGTAGAAGG-3'
#1867	5'-GCACAATACGAAGTCACTTTTCACC-3'

Peak28a (244 bp)

#2513	5'-CGTTACGGAGCTTTTGATCG-3'
#3028	5'-GGGAGTAACGATCAACCTTCG-3'

Peak28b (214 bp)

#3029	5'-CCGGACAGGTACATAGGTAC-3'
#2516	5'-AACCTCTGTCAAGGTTGACG-3'

Peak33a (221 bp)

#3019	5'-CGACGGAACAAATTACTACTATACACAAC-3'
#3020	5'-GGTTTTCGTATAGTAAGTTACCCGCTTC-3'

Peak33b (251 bp)

#3021	5'-AGGTTTTTGTTGTGTGCAGATG-3'
#3022	5'-AGGATGGCTGCAAGGCAGAAC-3'

cenVIIL (261 bp)

#2539	5'-CTTCTACTAGACCTAAGGGAGG-3'
#2550	5'-CGTCTTTGATAGTCGGGGATAAGG-3'

cenVIIR (294 bp)

#2541	5'-GGAGGTATAGAGGTACTAGGAG-3'
#2562	5'-CTTATTAAAGGTGCCCAGATATAATAGTAG-3'

Centromere markers

cenIIIL

#3725	5'-GGTCCCTGTATAAGAAGGTGG-3'
#3726	5'-GCTCCTTCAATATCATCTTCTGTCGACGGCGATCTCTAGGAAGGGTTCG-3'
#3727	5'-AGCTTTGTTCCCTTTAGTGAGGGTTAATTACTTTGAGCTGCCGGTATCC-3'
#3100	5'-AGTCCTCGGGGTACTAAAGC-3'

cenVIR

#3113	5'-GGAAAAGCCCTACTAGCACC-3'
#3728	5'-GCTCCTTCAATATCATCTTCTGTCGACGGCTGAATCTTCGATATCCGAC-3'
#3729	5'-CTTTGTTCCCTTTAGTGAGGGTTAATTGAGGAAATCATCCTTGAAGAGG-3'
#3730	5'-ATCTACGCGATCTCGGAGAC-3'

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 *Spe*I and *Bam*HI. pMF272[1] 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 pMF334[2], 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 *Not*I and *Pac*I. pMF270[3] was digested with *Not*I and *Pac*I 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 described[4].

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 genes[4]. 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 Δ*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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