SUPPLEMENTARY INFORMATION

The Cullin-4 complex DCDC does not require E3 ubiquitin ligase elements to control heterochromatin in Neurospora

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COP9 signalosome and DNA methylation

The COP9 signalosome (CSN) is believed to regulate the ubiquitin ligase activity of cullin complexes by removal of NEDD8 (1-3). Persistance of neddylation in Neurospora signalosome mutants (*csn*) results in autoubiquitination and degradation of the CUL1/SKP^{FWD1} complex (4). We wished to determine if a similar persistance of NEDD8 on CUL4 in the *csn* mutants could lead to autoubiquitination and degradation of DCDC, leading to loss of DNA methylation. However, we observed normal DNA methylation in *csn* mutants (Fig. S4). Consistent with this observation, proteomic analysis of purified epitope-tagged DCDC (DIM-8-FLAG) from a *csn* mutant revealed the presence of all DCDC components (Table S1). Treatment with cycloheximide inhibits protein synthesis and affects the stability of DCDC components CUL4 and DDB1in *csn* mutants (5). Again, we observed normal DNA methylation in cycloheximide treated *csn* mutant (Fig. S4), suggesting that DNA methylation may not require the continuous presence of DCDC at regions of DNA methylation.

Histone H2A ubiquitination is not required for DNA methylation in Neurospora

Recently, it has been speculated that monoubiquitination of histone H2A lysine 119 (H2AK119ub) by CRL4B may direct DNA methylation in mammals (6). H2A monoubiquitination by BRCA-1 also mediates heterochromatin formation (7-9). To explore the possibility that H2A is a substrate for DCDC, we expressed epitope-tagged H2A (wildtype and putative ubiquitination site mutant) in wildtype and *cul4* strains but failed to detect ubiquitination in any of the transformants by Western blotting. We also developed a method to generate *in vivo* substitutions in H2A and constructed Neurospora strains that have all H2A with the potential ubiquitination site mutated (Supplementary Methods and Fig. S5). DNA methylation was unaffected by H2AK122A substitution (Fig. S5). Similarly, DNA methylation was not affected by mutations in Neurospora genes encoding proteins that are known to ubiquitinate H2A for DNA repair in mammals (10-12), namely MMS2, RAD5, RAD6, RAD18 and RNF8 (Fig. S6).

SUPPLEMETARY METHODS

cul4 constructs

We constructed plasmids to complement the *cul4* mutant with the wildtype *cul4*$^{+}$ gene (with its native promoter and downstream regions; plasmid pKA67) or *flag-cul4*$^{+}$ (pKA122; this and similar constructs include Met-FLAG-5XGly-HAT-5XGly-3XFLAG-5XGly-CUL4, abbreviated as FLAG-CUL4 for simplicity) (13). To generate the *flag-cul4*^{K863A} construct we used pKA122 as template with primers 2286 and 2287 in the PCR-based QuickChange^{TM} Site-Directed Mutagenesis protocol (Stratagene) to generate plasmid pKA124 (*flag-cul4*^{K863A}). PCR-driven overlap extension was used to generate pKA287 (*cul4*^{K863A}) and pKA288 (*cul4*^{K863R}) (14). Briefly, genomic DNA from wildtype strain (N150) was used as template in PCR reactions with the following primers: *cul4*^{K863A} (primers 2193, 2194, 2286 and 2287) and *cul4*^{K863R} (primers 2193, 2194, 3417 and 3418). The PCR products were sequenced to confirm the presence of mutations and then cloned into the *Apa*I and *Spe*I site of pBM61 (15), to generate pKA287 and pKA288. To construct pKA294 (*flag-cul4*^{K863R}), pKA288 (*cul4*^{K863R}) was digested with *Not*I and *Nru*I to isolate a 581 bp fragment containing the mutation and this fragment was used to replace the corresponding region of *cul4* gene in pKA124. Plasmid pKA294 was digested with *Dra*I and plasmids pKA124, pKA287, pKA288 were digested with *Nde*I and targeted to the *his-3* locus of strain N3892 by electroporation (15). His^{+} transformants were selected for each construct (Table S2). Primers 1664 and 2201 were used to amplify a part of the *cul4* gene from the *his-3* locus of these His^{+} strains and the PCR products were sequenced to reconfirm the presence of desired mutations.

We also introduced the neddylation-site mutant alleles into a *cul4* deletion mutant. Primers 2193 and 2194 were used in PCR reaction with plamids pKA67 (13) (*cul4*$^{+}$), pKA287 (*cul4*^{K863A}) or pKA288 (*cul4*^{K863R}) as template to amplify wildtype or mutant alleles. The gene coding for the positive selectable marker *bar* was obtained by PCR using plasmid pBARKS1 (16) as template with primers 1652 and 1653. The PCR products for wildtype or neddylation-site mutant allele and *bar* were co-transformed into the *cul4* deletion mutant (N3169) by electroporation (15). Multiple Bar^{+} transformants

were selected for each of the *cul4* alleles and their DNA methylation was analyzed by Southern blotting (Fig. S3).

To generate *flag-cul4*$^{1\text{-}823}$, genomic DNA from a wildtype strain (N150) was used as template in PCR reactions with primers JGP1 and JGP3. The PCR product was then cloned into the *BglII* and *XbaI* sites of pKA122, to generate pJG1. To generate *cul4*$^{1\text{-}823}$, genomic DNA from a wildtype strain (N150) was amplified using primers JGP52 and JGP54 and the PCR product was cloned into the *ApaI* and *SpeI* sites of pKA67 to generate pJG2. Plasmids pJG1 and pJG2 were digested with *NdeI* and targeted to the *his-3* locus of strain N3892 by electroporation. His^{+} transformants were isolated for each construct and made homokaryotic through microconidiation.

rbx1 deletion mutant

To generate a knockout mutant for the putative *rbx1* gene (NCU11300), fragments corresponding to the 5' UTR and 3' UTR were PCR amplified with primers JGP158/JGP159 and JGP162/JGP163 respectively using genomic DNA from a wild-type strain (N150) as template. These fragments also contained homology to the *PtrpC-hph* (hygromycin resistance) cassette (17). Additionally, a fragment containing *PtrpC-hph* and homology to the 5' and 3' UTR regions of NCU11300 was amplified with primers JGP160 and JGP161 using plasmid *p3xFLAG::hph*$^{+}$*::loxP* (17) as template. To generate a knockout cassette for the *rbx1* gene (*NCU11300 5' UTR-PtrpC::hph*$^{+}$*-NCU11300 3'UTR*), these three fragments were used as template in an overlap-extension PCR reaction using primers JGP158 and JGP161. This knockout cassette was transformed into strain N2930 by electroporation (15). Replacement of the native *rbx1* gene with this hygromycin resistance cassette generated Hyg^{R} transformants that were purified by microconidiation. We were unable to obtain homokaryotic transformants, suggesting that *rbx1* gene is essential. However, we were successful in enriching the nuclei harboring the deletion using the antibiotic (hygromycin) selection of microconidia; this

enrichment was verified by Southern blotting using a probe corresponding to the 3' UTR region of NCU11300 (Fig. S7).

hH2A constructs

The construction of epitope tagged H2A strains is breifly described here. Wildtype (N150) genomic DNA was used as template in PCR with primers 2338 and 2686 to amplify a 2.1 kb fragment containing the *hH2A* gene and 1.4 kb of 5' UTR. This PCR product was cloned into the *Not*I and *Eco*RI sites of a plasmid (pRS416+10XGly) (17) to generate plasmid pKA130. Plasmid pKA130 was digested with *Not*I + *Pac*I and the 2.1 kb fragment was subcloned between the *Not*I and *Pac*I sites of pCCG::C-Gly::3XFLAG (17) to obtain plasmid pKA133. In pKA133, the *hH2A* gene is separated from the 3XFLAG epitope by a 10XGly spacer and the expression of H2A-FLAG is driven by the native promoter. To change the conserved ubiquitination site (K122; Fig. S5), PCR-driven overlap extension was used with primers 2271, 2272, 2338 and 2686 and plasmid pKA130 as template (14). After confirming the mutation by sequencing, the PCR product was cloned between the *Not*I and *Eco*RI sites of a plasmid (pRS416+10XGly) (17) to generate pKA137. Plasmid pKA137 was digested with *Not*I and *Pac*I and the 2.1 kb fragment was subcloned between the *Not*I and *Pac*I sites of pCCG::C-Gly::3XFLAG (17) to generate pKA139. Plasmid pKA133 was digested with *Xmn*I and a 7.1 kb fragment containing the epitope-tagged *hH2A* gene along with the flanking *his-3* sequences was gel-purified and targeted to the *his-3* region of the host strain by electroporation. Several His+ transformants were analyzed by Southern blotting (to confirm correct integration) and Western blotting (to detect H2A-FLAG) and strain N4197 was selected for further experiments. Similarly strain N4201 (expressing $H2A^{K122A}$-FLAG) was constructed using plasmid pKA139.

In order to replace the endogenous *hH2A* gene with inl^{+}, we constructed plasmid pKA120 as follows. Genomic DNA from wildtype strain (N150) was used as template in PCR reactions with primers 2318 and 2319 to amplify 1.4 kb of 5' UTR and primers 2320 and 2321 to amplify 1 kb of 3'

UTR of the *hH2A* gene. The *inl* (myoinositol-1-phosphate synthase) plasmid pOKE1 was used as template with primers 1497 and 1498 to amplify a 2.6 kb fragment containing the wildtype inl^+ gene. These three PCR products were mixed with DNA of linerized pRS416 (digested with *Bam*HI and *Eco*RI), and transformed into yeast strain PJ49-6A (18). Plasmid DNA was isolated from ura^+ colonies and transformed into *E. coli* strain *DH5αF′*. Plasmid pKA120 was isolated from a transformant, digested with *Bam*HI and *Eco*RI and the 5.1 kb fragment was purified and transformed into a *inl*, *mus-52* strain (N2993) by electroporation (15). To confirm correct integration, 15 Inl^+ transformants were analyzed by Southern hybridization and strain N4195 was selected for further study.

To obtain strains expressing only H2A-FLAG, strain N4197 was crossed with N4195 and progeny growing on minimal medium were screened by Southern hybridization to obtain strains N4528 and N4529. Similarly, to obtain strains expressing only $H2A^{K122A}$-FLAG, strain N4201 was crossed with N4195 and progeny growing on minimal medium were screened by Southern hybridization to obtain strains N4533 and N4534.

TABLE S1. Mass-spectroscopic analyses of DCDC from CUL4 neddylation-site and signalosome mutants[1].

			DIM-9-FLAG	DIM-8-FLAG	
			WT	*cul4*^{K863A}	*csn-1*
Locus[2]	**Description**	**MW[3]**	**Peptides[4]**	**Peptides[4]**	**Peptides[4]**
NCU06605	DIM-8/DDB1	128.6	72	216	181
NCU00272	CUL4	112.9	54	160	73
NCU01679	WD40 protein	68.97	0	74	19
NCU01656	DIM-9 (WD40 protein)	136.7	70	40	42
NCU01290	CBF5	52.8	0	40	0
NCU03668	WD40 protein	88.1	0	25	5
NCU04152	DIM-7	74.2	22	19	22
NCU04799	PABP	82.3	0	15	1
NCU02151	WD40 protein	75.9	0	14	13
NCU06598	Fungal specific protein	103.6	7	12	2
NCU08951	H/ACA ribonucleoprotein complex subunit 2	26.8	0	10	1
NCU07735	Grp1p	37.8	0	6	0
NCU01197	Ssd1	148.3	0	6	0
NCU06459	Negative regulator of differentiation 1	85.7	0	5	0
NCU06210	60S ribosomal protein L28e	159.8	0	3	0
NCU04402	DIM-5	37.5	1	2	0
NCU01634	Histone H4	11.3	0	2	0
NCU02437	Histone H2A	14.1	0	2	0
NCU05513	RCC-1	65.6	0	2	0
NCU05347	Histone H2A.Z	15.3	0	1	0
NCU03309	NEDD-8	8.8	4	0	1

[1] DIM-8-FLAG was purified from a CUL4-neddylation site mutant (*cul4*^{K863A}) or a COP9 signalosome mutant (*csn-1*). Purification of DIM-9-FLAG from a wildtype strain recovered all DCDC members (highlighted in red), similar to identification of DCDC with FLAG-CUL4 (13). Strains are listed in Supplementary Table 2.

[2] http://www.broadinstitute.org/annotation/genome/neurospora/MultiHome.html

[3] kDa

[4] Number of peptides.

TABLE S2. Strains used in this study

Strain	Genotype	Used in Figure/Table	Source
N150	74-OR23-1 V *mat A*	1C,D;2A,B,C;3A, B;S2-7	Our lab
N623	*his-3 mat A*		Our lab
N625	*his-3 mat a*		Our lab
N3016	*Sad-1 his-3 mat a*		Our lab
N1860	*dim-2*	S5,S6	Our lab
N3892	*Sad-1 his-3; cul4^{RIP1} mat a*	1B,C,D;2A,B,C;3 A,B;S2-3	
N3893	*Sad-1 his-3^{+}::cul4 $^{+}$; cul4^{RIP1} mat a*	1D;2B,C;3A;S2	
N3894	*Sad-1 his-3^{+}::cul4 $^{+}$; cul4^{RIP1} mat a*	S2	(13)
N3896	*Sad-1 his-3^{+}::flag-cul4^{+}; cul4^{RIP1} mat a*	1B,C	
N3898	*Sad-1 his-3^{+}::flag-cul4^{K863A}; cul4^{RIP1} mat a*	1B,C	This study
N4286	*Sad-1 his-3^{+}::flag-cul4^{K863R}; cul4^{RIP1} mat a*	1B,C	This study
N4279	*Sad-1 his-3^{+}::cul4^{K863A}; cul4^{RIP1} mat a*	1D;S2	This study
N4280	*Sad-1 his-3^{+}::cull^{K863A}; cul4^{RIP1} mat a*	S2	This study
N4269	*Sad-1 his-3^{+}::cul4^{K863R}; cul4^{RIP1} mat a*	1D;S2	This study
N4270	*Sad-1 his-3^{+}::cul4^{K863R}; cul4^{RIP1} mat a*	S2	This study
N3169	*cul4^{Δ}::hph^{+} mat a*	S3	FGSC#12374
N2930	*his-3 mus-52^{Δ}::bar^{+}; mat A*		Our lab
N2993	*his-3 mus-52^{Δ}::bar^{+}; inl^{Δ} mat A*		Our lab
N4195	*his-3 mus-52^{Δ}::bar^{+}; inl^{Δ}; hH2A^{Δ}::inl^{+} mat A*		This study
N4197	*his-3^{+}::hH2A-FLAG; Sad-2^{Δ}::hph^{+} inl^{Δ} mat a*		This study
N4201	*his-3^{+}::hH2A^{K122A}-FLAG; Sad-2^{Δ}::hph^{+} inl^{Δ} mat a*		This study
N4528	*his-3^{+}::hH2A-FLAG; hH2A^{Δ}::inl^{+}*	S5	This study
N4529	*his-3^{+}::hH2A-FLAG; hH2A^{Δ}::inl^{+}*	S5	This study
N4533	*his-3^{+}::hH2A^{K122A}-FLAG; hH2A^{Δ}::inl^{+}*	S5	This study
N4534	*his-3^{+}::hH2A^{K122A}-FLAG; hH2A^{Δ}::inl^{+}*	S5	This study
N5112	*csn-1^{Δ}::hph^{+}*	S4	FGSC#11281
N5113	*csn-1^{Δ}::hph^{+}*	S4	FGSC#11282
N5029	*his-3^{+}::cul4^{K863A}; cul4^{RIP1}; dim-8-flag::loxP::hph^{+}::loxP*	Table S1	This study
N3970	*dim-9-flag::loxP::hph^{+}::loxP*	Table S1	This study
N5030	*csn-1^{Δ}::hph^{+}; dim-8-flag::loxP::hph^{+}::loxP*	Table S1	This study
N5292	*Sad-1 his-3^{+}::flag-cul4$^{1-823}$; cul4^{RIP1} mat a*		This study
N5299	*Sad-1 his-3^{+}::cul4$^{1-823}$; cul4^{RIP1} mat a*	2B,C,D	This study
N4616	*mms2^{Δ}::hph^{+}*	S6	This study
FGSC15992	*rnf8^{Δ}::hph^{+}*	S6	FGSC
	mus-41, rad-5b,-c	S6	(19)
FSSC11445	*uvs-2^{Δ}::hph^{+}*	S6	FGSC
FGSC15992	*upr-1^{Δ}::hph^{+}*	S6	FGSC
N5523	*rbx1^{Δ}::hph^{+}his-3 mus-52^{Δ}::bar^{+}; mat A*	3A,B;S7	This study

TABLE S3. Primers used in this study.

Primer	Sequence
1652	CCGTCGACAGAAGATGATATTGAAGGAGC
1653	AATTAACCCTCACTAAAGGGAACAAAAGC
1654	AGCTGACATCGACACCAACG
1664	ATTCAGACCCCATTAGCCGTCCACGCC
2193	CGCTTAAGAGCGCTGACATAGCGATGATGCTT
2194	GCTCTAGAGCCATTCGTTAGAATCACAATACA
2201	GCGCGTCCTCAACAAACACCC
2286	GTGCGCATCATGGCCAGCCGGAAGAAG
2287	CTTCTTCCGGCTGGCCATGATGCGCAC
3417	GTGCGCATCATGCGCAGCCGGAAGAAG
3418	CTTCTTCCGGCTGCGCATGATGCGCAC
2338	ATAAGAATGCGGCCGCGCCTGATCGCTGATGGGA
2686	CGGAATTCAAGTTCTTGACTCGCGTT
2271	CTCCTTCCTAAGGCCACCGGCAAGACT
2272	AGTCTTGCCGGTGGCCTTAGGAAGGAG
2318	GTAACGCCAGGGTTTTCCCAGTCACGACGGGATCCGCCTGATCGCTGATGGGA
2319	GTGGATGCACGTCTGCCCTGGAGATTGTTGGAGCTCTAAGACTCGGAAGTCGCG
2320	TGGTGGTTGTTGACCGTGATACAAACTCCTAGCTAGCCTGTATAAAGTAACGATA
2321	TGAGCGGATAACAATTTCACACAGGAAACAGCGAATTCGTACCTAGTGGAGTGAAC
1497	GCCGAGCTCCAACAATCTCCAGGGCAGACG
1498	GCCGCTAGCTAGGAGTTTGTATCACGGTCA
2852	GTAACGCCAGGGTTTTCCCAGTCACGACGAAAGGTCAGGGGCGTTGA
2853	ACCGGGATCCACTTAACGTTACTGAAATCTGTTTCTTAGTACTTTTG
2854	GCTCCTTCAATATCATCTTCTGTCGACGGACATCTACCGGTACCGGA
2855	GCGGATAACAATTTCACACAGGAAACAGCGCTTCATTGCTTCATTGC
hph-FP	CCGTCGACAGAAGATGATATTGAAGGAGC
hph-RP	AGCTGACATCGACACCAACG
JGP1	CGATGCGAGATCTATGGCCACGGGCAAAACACCA
JGP3	CGATGCGTCTAGAGAACTTGGGATCGGTGAAGGA
JGP52	CGATGCGACTAGTAACGGCCGCCAGTGTGCTGGA
JGP54	CGATGCGGGGCCCCTAGAACTTGGGATCGGTGAA
JGP158	AGCAAGGCGGCGATAAGGGGT
JGP159	TTCTGTCGACTTCAACTGTCTTTTTGAATCTCTCGTTCG
JGP160	GACAGTTGAAGTCGACAGAAGATGATATTGAAGG
JGP161	TACCGCGGAACTACTCTATTCCTTTGCCCTCGG
JGP162	AATAGAGTAGTTCCGCGGTACTTGCTAGGG
JGP163	GTGGGTCCTAGAAGGGAACTAGCT

N. crassa	CUL4	854	TQAAIVRIMKSRKKMAHAQL	873
S. pombe	Pcu4	671	LQASIVRVMKQKEKMKHDDL	690
C. elegans	Cullin 4	777	IDAAVVRIMKARKQLNHQTL	896
D. melanogaster	Cullin 4	758	IDAAIVRIMKMRKTLSHNLL	777
A. thaliana	Cullin 4	679	IDAAIVRIMKTRKVLSHTLL	698
H. sapiens	CUL-4A	696	IDAAIVRIMKMRKTLGHNLL	715
H. sapiens	CUL-4B	749	IDAAIVRIMKMRKTLSHNLL	768

Fig. S1. NEDD8 attachment site is conserved in CUL4. Alignment of a conserved region in the carboxy termini of Cullin 4 proteins from various eukaryotes including *Neurospora crassa* (13), *Schizosacchromyces pombe* (BAA32520, accession number at NCBI), *Caenorhabditis elegans* (AAA68791), *Drosophila melanogaster* (NP_001163084), *Arabidopsis thaliana* (CAC85265) and *Homo sapiens* (CUL-4A: Q13619 and CUL-4B: Q13620). Identical residues are colored blue, NEDD8 is attached to the lysine that is indicated in red.

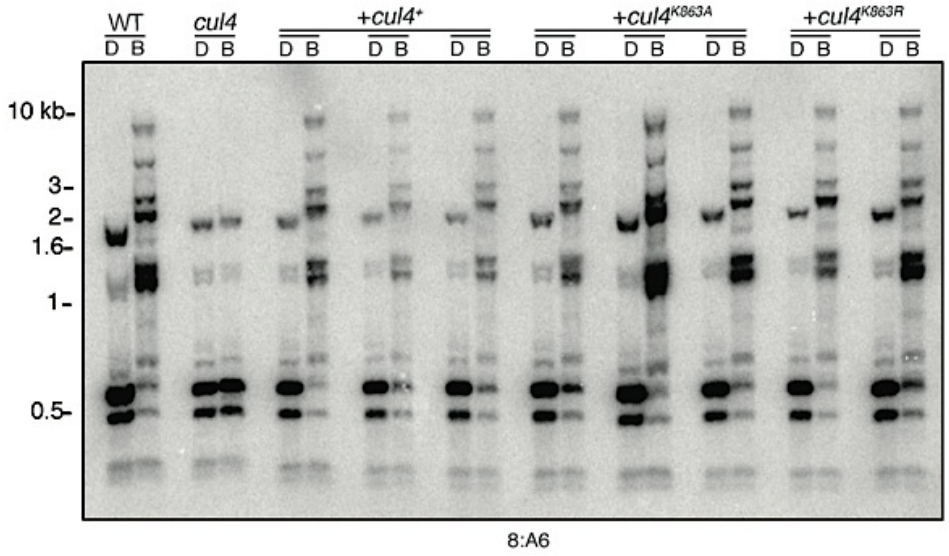

Fig. S2. DNA methylation analysis of wildtype (WT), *cul4* and transformed *cul4* strains expressing the indicated untagged CUL4 constructs. DNA was digested with 5mC-sensitive *Bfu*CI (B) or its 5mC-insensitive isoschizomer *Dpn*II (D) and the Southern blot was probed for methylated region 8:A6 (20); kb size standards (left). Equivalent results were obtained for other methylated regions (5:B8). Strains are listed in Table S2.

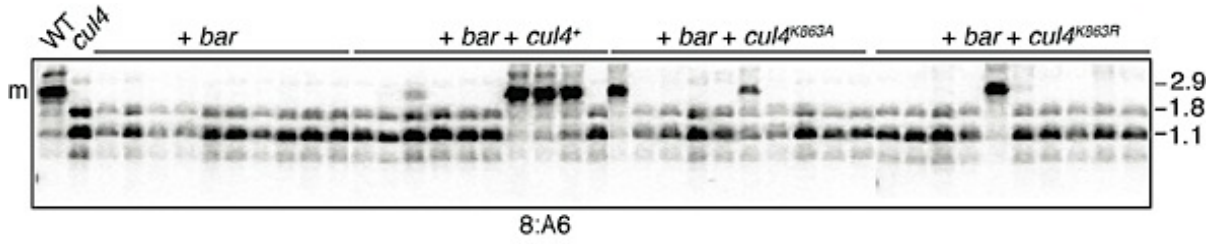

Fig. S3. *cul4* neddylation-site mutant alleles complement DNA methylation in a *cul4* deletion mutant. A *cul4* deletion mutant (N3169) was transformed by electroporation (15) with a PCR product encoding basta resistance (*bar*) or co-transformed with PCR products encoding basta resistance (*bar*) and wildtype ($cul4^{+}$) or neddylation-site mutant ($cul4^{K863A/R}$) alleles (Supplementary Methods). DNA from multiple Bar^{+} transformants were digested with 5mC-sensitive *Ava*II and the Southern blot was probed for methylated region 8:A6 (20). Methylation of *Ava*II site prevents digestion in wildtype strain (2.9 kb band labeled 'm'), while lack of DNA methylation in *cul4* mutant permits digestion by *Ava*II resulting in two fragments (1.8 and 1.1 kb). Transformation with either wildtype ($cul4^{+}$) or neddylation-site mutant alleles ($cul4^{K863A/R}$) resulted in complementation.

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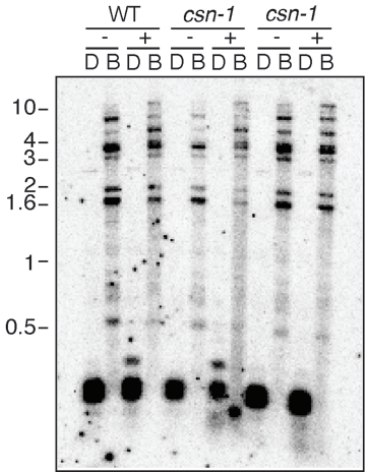

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191 **Fig. S4. COP9 Signalosome (CSN) is not required for DNA methylation.** Normal DNA methylation

192 in *csn-1* mutant. DNA from wildtype strain (N150) or two *csn-1* strains was digested with *Bfu*CI (B) or

193 *Dpn*II (D) and the Southern blot was probed for methylated region 8:A6 (20); kb size standards (left).

194 Cultures were grown in absence (-) or presence of 10 μg/ml cycloheximide (+). Strains are listed in

195 Table S2.

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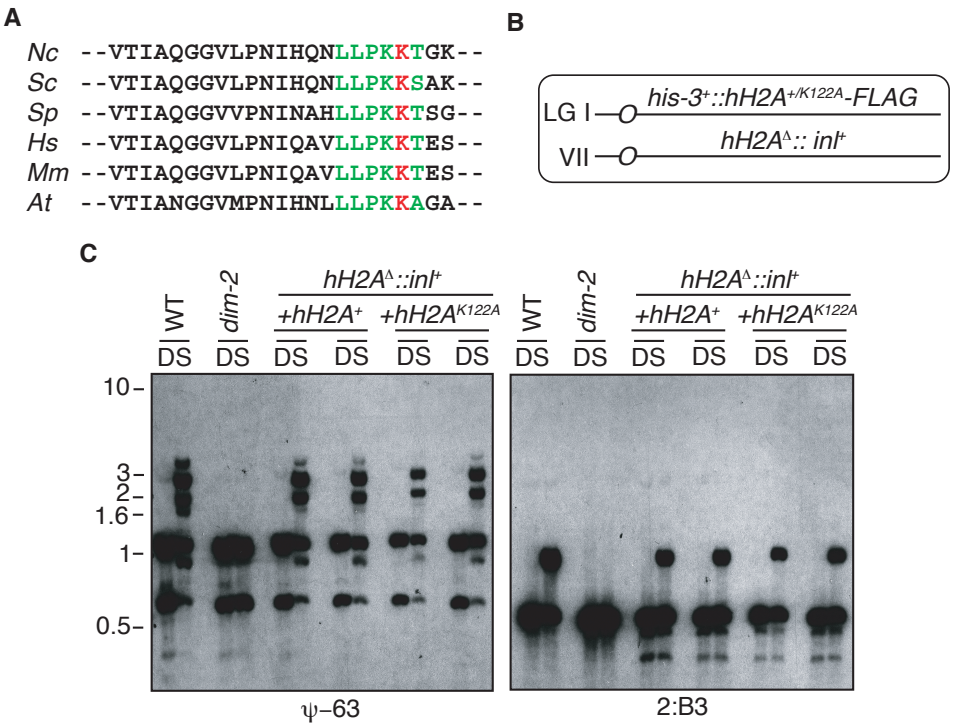

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201 **Fig. S5. Histone H2A ubiquitination is not required for DNA methylation in Neurospora.** (A)
202 Alignment of H2A C-terminal tails from various eukaryotes including *Neurospora crassa* (21),
203 *Saccharomyces cerevisiae* (CAA81267.1; accession number at NCBI), *Schizosaccharomyces pombe*
204 (NP_588180.1), *Homo sapiens* (NP_003502.1), *Mus musculans* (CAA83210.1) and *Arabidopsis*
205 *thaliana* (NP_200275.1). The conserved recognition sequence is highlighed in green and the conserved
206 site that is ubiquitinated in some organisms is red. (B) Genotype of strains expressing epitope-tagged
207 wildtype (H2A-FLAG) or ubiquitination site mutant (H2AK122A-FLAG). The endogenous gene was
208 replaced with a selectable marker (*inl*$^{+}$). (C) DNA methylation is normal in the H2AK122A mutant.
209 DNA from wildtype (WT), *dim-2* mutant and strains of indicated genotypes was digested with *Sau*3AI
210 (S) or *Dpn*II (D) and the Southern blot was probed for methylated regions Ψ-63 and 8:G3 (20); kb size
211 standards (left). Absence of DNA methylation in the *dim-2* (22) mutant results in identical pattern for
212 *Dpn*II and *Sau*3AI. Strains are listed in Table S2.

Adhvaryu_et._al._Fig. S6

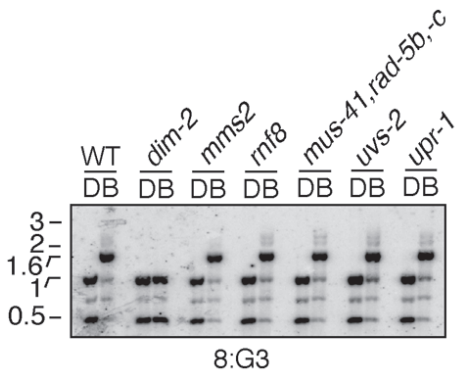

Fig. S6. DNA methylation is normal in various DNA repair mutants. DNA was digested with *Bfu*CI (B) or *Dpn*II (D) and the Southern blot was probed for methylated region 8:G3 (20); kb size standards (left). Strains are listed in Table S2.

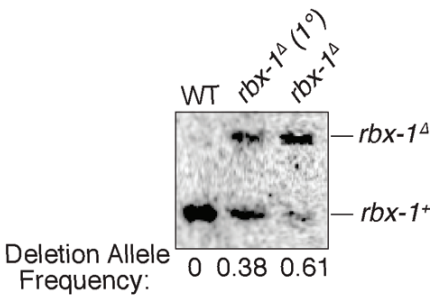

Fig. S7. Generation of *rbx1* deletion mutant. The endogenous allele was replaced with a hygromycin resistance selection cassette (Supplementary Methods) and the primary transformant (1°) was purified by microconidiation. The fraction of nuclei bearing the deletion was assessed by comparing the relative intensity of bands for the deletion and endogenous allele. The *rbx1* gene appears to be essential and we were unable to obtain a homokaryon. Strains are listed in Table S2.

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