Supporting Information

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SI Materials and Methods

Plasmid Construction. DIM-7 CDS FP and RP plus DIM-7 UTR FP and RP primers were used to amplify the *dim-7* coding sequence and 3′UTR, respectively, to make the knock-in 3XFLAG-tagged construct as described (1). DIM-7-NotIFP and DIM-7-PacI-RP were used to amplify *dim-7* from genomic DNA and clone this construct into the pCCG::C-3XFLAG plasmid digested with NotI and PacI. pCCG::C-3XFLAG includes a 3XFLAG tag and sequences for *his-3* targeting to create a DIM-7–3XFLAG plasmid. We note that the *ccg-1* promoter has been deleted and the DIM-7–3XFLAG construct is under control of its native promoter. DIM-7-XbaI-FP and DIM-7-PacI-RP were used to amplify most of the coding sequence into a pCR2.1 vector (Invitrogen) to generate the DIM-7-Xba-Pac plasmid for constructing mutant versions of the DIM-7 protein. DIM-7 deletion mutants were obtained by performing inverse PCR using delta NT domain primers P1 and P2, or delta ZF primers P1 and P2, and the DIM-7-Xba-Pac plasmid as a template. The resulting linear PCR product was subject to blunt end ligation and transformed into *Escherichia coli* to generate the plasmid containing a mutant, truncated DIM-7 sequence. The DIM-7 C579G mutant was constructed by performing Quickchange (Stratagene) site directed mutagenesis using the C579G P1 and P2, plus the DIM-7-Xba-Pac plasmid as a template. The mutated *dim-7* sequences were then subcloned into the DIM-7–3XFLAG plasmid using the XbaI and PacI sites to generate full-length, FLAG-tagged, mutant DIM-7 constructs.

The *his-3* FP and RP primers, *his-3*flank fp and rp primers, and the *dim-5*-his3_fp and *dim-5*_gly_rp primers were used to amplify *his-3* coding sequence, *his-3* flanking sequence, and *dim-5,* respectively, from genomic DNA. *E. coli* Dam was amplified from plasmid pCmyc-Dam (http://research.nki.nl/vansteensellab/DamIDinfo/DrosophilaDamID/Plasmids/Drosophila_plasmids.htm) using C_dam_fp and C_dam_hisflank_rp primers. A plasmid containing the DIM-5-Dam fusion with *his-3* targeting sequences was assembled by yeast in vivo recombination with pRS416 as described (2).

1. Honda S, Selker EU (2009) Tools for fungal proteomics: multifunctional neurospora vectors for gene replacement, protein expression and protein purification. *Genetics* 182(1):11–23.

2. Colot HV, et al. (2006) A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103: 10352–10357.

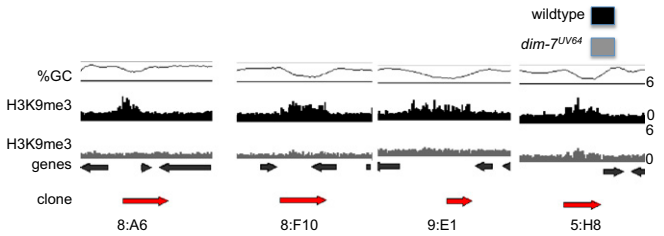

Fig. S1. H3K9me3 enrichment at previously identified methylated regions. ChIP-chip data from four representative control regions are shown [Selker EU, et al. (2003) The methylated component of the *Neurospora crassa* genome. *Nature* 422:893–897]. The base composition (%GC) is plotted as the moving average at the top of each plot. ChIP-chip data for WT (black) and the *dim-7*^{UV64} strain (gray) are shown as enrichment values [H3K9me3 IP/input] indicated on the *y* axis. The positions of predicted ORFs (genes) and the previously identified methylated DNA clones (red), with their identification numbers, are shown at the bottom.

A

Mass: 36003.73018 **pI:** 6.08 **Coverage:** 40.57%

EKAFRPHFF NHGKPDIRSF ATHAQLPISI VNREDDAFLN PNFRFIDHSI IGKNVPVADQ SFRVGCSCAS DEECMYSTCQ CLDEMAPDSD EEADPYTRKK
FAYYSQGAK KGLLRDRVLQ SQEPIYECHQ GCACSKDCPN RVVERGRTVP LQIFRTKDRG WGVKCPVNIK RGQFVDRYLG EIITSEEADR RRAESTIARR
DVYLFALDK FSDPDSLDPL LAGQPLEVDG EYMSGPTRFI NHSCDPNMAI FARVGDHADK HIHDLALFAI KDIPKGTELT FDYVNGLTGL ESDAHDPSKI
EMTKCLCGT AKCRGYL

B

Mass: 74193.0550800001 **pI:** 8.02 **Coverage:** 31.47%

MAGPGRPRRR RGSSASIDST DDRRRRWTRE LAILKPVTPG TSDDLWPYFA VLTDATIYQK DGKTLANPLH VDLEGPFIVR GKLEPVEDGD DEARECFHKP
YNKATYIEIS RSERYSIGYE PNTLWVSGAS GWFEIIPSRK YETMYNEVME AITLYYGIMG PYEEHKRLLK KADKKKRKDV KPPSLDEIFF SYALTAGDGA
VTVKEEVEAR CLKWAQFLLA HFPKESEIRW QDTGFAAWLQ SARPDLQKKI SDVAAGLLTK PAAEPEGLLA RDDHSPQPQS LAIREPPKGG RTTRTSRQAS
ELSDQRSDKG KGIARDSPVE TPIPAPVSNR HLSSSSPRPR ASPAVITGSD DLPDDPVDRL IVLLGEVAQR IDIKTVKMSK INSDLFYNCR IKYYNAAREL
CEHFAKDLLE RLPPMWDNTP YRAWLEDVVE NGRPAPVEFV VEDIPQYLTR RTRRQTHISR LSSALGPTPS VEAESKDEVR AGKQPRVGRP SGKVATLRVA
GSKRLASDMI DEDELPPRRG RKALKRTVRV EEDEDSDANS VIIDMTDNTG VIPKDAVRIV VQAERLPTMS PAGPNGTWTC DQEGCNYVVR SADEPEGQDL
ISKHFKDHEE QAQKINLAME ESSHSHLLDK IQAMGKNALA KKRGSLNGEP LPLPIKRRLL

Fig. S2. Peptide coverage obtained for DIM-5 and DIM-7. Peptides identified by mass spectrometry following purification of DIM-5 are shown highlighted in red on the primary amino acid sequence for (*A*) DIM-5 and (*B*) DIM-7. Complete proteomic analysis of DIM-5-interacting proteins and detailed methods will be described elsewhere. The predicted molecular weight and isoelectric point, as well as the percent of the total protein covered by the identified peptides, is shown.

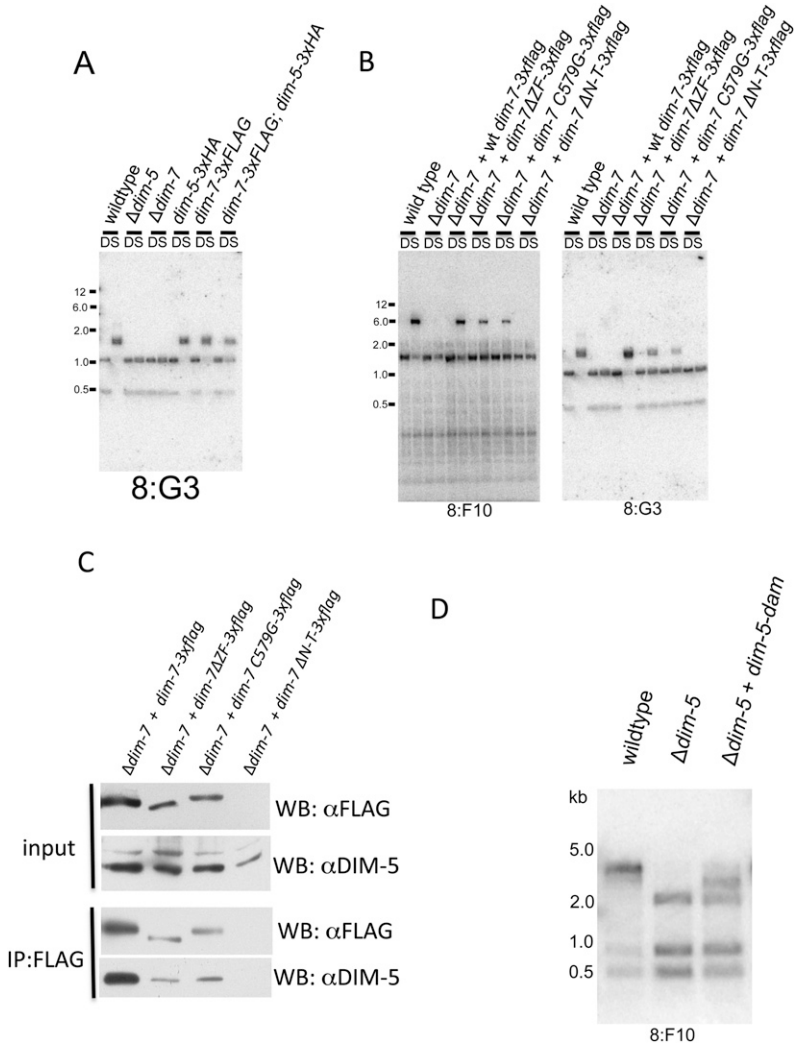

Fig. S3. DNA methylation and DIM-5 interaction of WT and mutant FLAG-tagged DIM-7 fusion proteins. (*A*) Southern blot showing that strains expressing DIM-5–3XHA, DIM-7–3XFLAG, or both proteins from the endogenous *dim-5* and *dim-7* loci, respectively, carry out DNA methylation at the 8:G3 region, indicating that the tagged proteins are functional. (*B*) Southern blot depicting DNA methylation at the 8:F10 or 8:G3 regions in Δ*dim-7* strains expressing WT or mutant DIM-7 proteins, as indicated. The mutated regions/residues are indicated in Fig S4. (*C*) Western blots of input and immunoprecipitated fractions of Δ*dim-7* strains expressing wild type or mutant DIM-7 proteins. The input fraction and the anti-FLAG immunoprecipitate fraction (IP:αFLAG) were probed with the anti-FLAG or anti-DIM-5 antibodies as indicated (WB, Western blot). (*D*) Partial restoration of DNA methylation by expression of the DIM-5-Dam fusion in a *Δdim-5* strain. Genomic DNA from strain N150 (WT), strain N3074 (Δ*dim-5*), or strain N3863 (Δ*dim-5* + *dim-5-dam*) was digested using the cytosine methylation-sensitive, but adenine methylation-insensitive restriction endonuclease *AvaII*, fractionated by gel electrophoresis, transferred to a nylon membrane, and hybridized to a probe corresponding to the methylated 8:F10 region [Selker EU, et al. (2003) The methylated component of the *Neurospora crassa* genome. *Nature* 422:893–897].

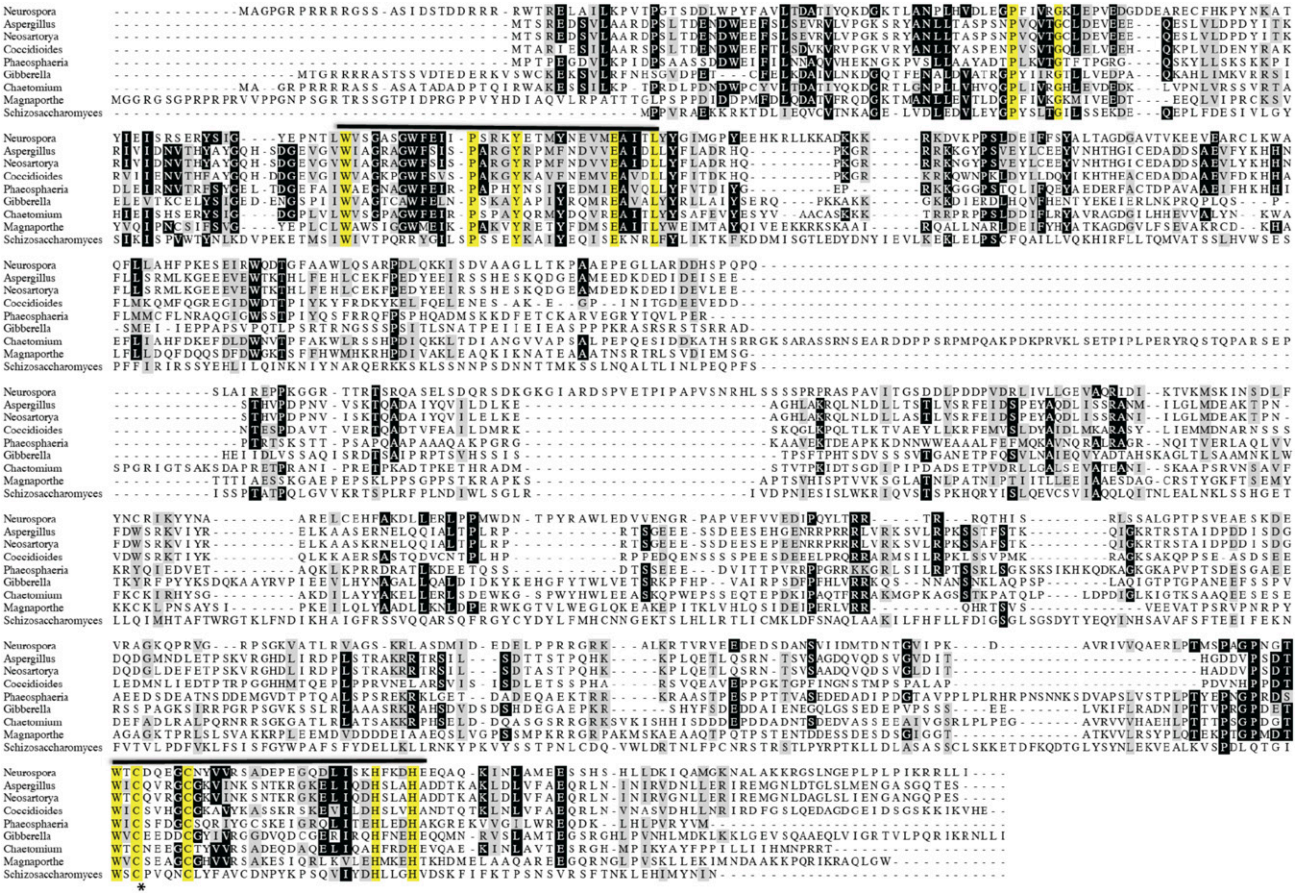

Fig. S4. Alignment of DIM-7 protein sequences from several filamentous fungi. ClustalW alignment of the predicted DIM-7 protein sequences from *Aspergillus fumagatis* (*Aspergillus*)(XP_001481513), *Phaeosphaeria nodorum* (Phaeosphaeria) (XP_001800529), *Gibberella zeae* (Gibberella)(XP_387503), *Chaetomium globosum* (Chaetomium)(XP_001224793), *Neurospora crassa* (Neurospora)(XP_961308), *Magnaporthe grisea* (Magnaporthe)(XP_363041), *Neosartorya fischeri* (Neosartorya)(XP_001259468), and *Coccidioides immitis* (Coccidiodes)(XP_001239633), plus Raf2p from *Schizosaccharoymyces pombe* (CAA20700). The region deleted in the N-terminal domain deletion allele (ΔNT) is indicated by a black line above the deleted sequence (W124–L153). The region deleted in the zinc finger-domain deletion allele (ΔZF) is also indicated by a black line above the deleted sequence (W577 to H607). An asterisk indicates Cysteine 579, which was mutated to a Glycine.

Table S1. Reversion frequency of selectable markers

Medium	Strain→ Number of conidia plated	N1674 Number of colonies*	N2984 Number of colonies*
No drug	10^5	Lawn†	Lawn†
	5 X 10^5	Lawn	Lawn
	5 X 10^6	Lawn	Lawn
	10^7	Lawn	Lawn
Hygromycin	10^5	2, 3, 1	1, 0, 1
	5 X 10^5	10, 8, 7	5, 8, 7
	5 X 10^6	80, 55, 70	55, 75, 65
Basta	10^5	No colonies*	0, 1, 3
	5 X 10^5	No colonies*	8, 10, 7
	5 X 10^6	No colonies*	40, 55, 48
Hygromycin + Basta	10^5	No colonies	0, 0, 0
	5 X 10^5	No colonies	0, 0, 0
	5X 10^6	No colonies	1, 0, 2
	10^7	No colonies	3, 4, 1

*Numbers separated by commas indicate the number of colonies/plate for three replicate plating experiments.
†Lawn indicates that a lawn of colonies was obtained when the indicated number of conidia were plated on nonselective medium (No drug).

Table S2. Strains used in the present study

Strain	Genotype	Reference
N150	Wild type	(1)
N1674	*am^{132} lys-1 inl;::am^{RIP}-hph^m-am^{RIP}*	(2)
N2984	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl;::am^{RIP}-hph^m-am^{RIP} a*	Present study
N3101	*ΔinlA*	Gift*
N2977	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} Δinl;::am^{RIP}-hph^m-am^{RIP} a*	Present study
N3074	*Δdim-5 trp-2*	(3)
N1851	*Δdim-2*	(4)
N3311	*Sad-1 his-3^{RIP};:bar^M-his-3^{RIP}; am^{132} Δinl;::am^{RIP}-hph^m-am^{RIP}A*	Present study
N3854	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl;::am^{RIP}-hph^m-am^{RIP} a ;: ;Bml;::dim-2kd*	Present study
N3312	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl, dim-7^{UV64};::am^{RIP}-hph^m-am^{RIP} a*	Present study
N3866	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl, dim-7^{UV30};::am^{RIP}-hph^m-$am^{R!P}$ a*	Present study
N3867	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl, dim-7^{UV68};::am^{RIP}-hph^m-am^{RIP} a*	Present study
N3868	*his-3^{RIP}::bar^M-his-3^{RIP}; am^{132} inl, dim-7^{UV64};::am^{RIP}-hph^m-am^{RIP} a;::Bml; ;;dim-7^+*	Present study
FGSC 14210	*Δdim-7::hph+ heterokaryon a*	(5)
N3855	*Sad-1 Δdim-7::hph+ A*	Present study
N3856	*Δdim-7::hph+ a*	Present study
N3857	*dim-7^+::dim-7-flag - hph*	Present study
N3316	*dim-5^+::dim-5–3×HA - hph*	Present study
N3858	*dim-7^+::dim-7–3×flag-hph ; dim-5^+::dim-5–3×ha-hph*	Present study
N3859	*his-3 $^+$::dim-7–3×flag; Adim-7*	Present study
N3860	*his-3 $^+$::dim-7ΔZF-3×flag; Adim-7*	Present study
N3861	*his-3 $^+$::dim-7C579G-3×flag; Δdim-7*	Present study
N3862	*his-3 $^+$::dim-7-ΔNT-3×flag; Δdim-7*	Present study
N3863	*his-3 $^+$::dim-5-dam ; Δdim-5::bar+*	Present study
N3864	*his-3 $^+$::dim-5-dam*	Present study

*Generously provided by Rodolfo Aramayo, Texas A&M University, College Station, TX.

1. Galagan JE, et al. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* 422:859–868.
2. Irelan JT, Selker EU (1997) Cytosine methylation associated with repeat-induced point mutation causes epigenetic gene silencing in *Neurospora crassa*. *Genetics* 146:509–523.
3. Lewis ZA, et al. (2009) Relics of repeat-induced point mutation direct heterochromatin formation in *Neurospora crassa*. *Genome Res* 19:427–437.
4. Kouzminova EA, Selker EU (2001) *dim-2* encodes a DNA methyltransferase responsible for all known cytosine methylation in *Neurospora*. *EMBO J* 20:4309–4323.
5. Colot HV, et al. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103:10352–10357.

Table S3. Primers used in the present study

Name	Sequence
DIM-7 CDS FP	GAGGTCGACGGTATCGATAAGCTTGATATGCAACCTCGGGTAGGCCG
DIM-7 CDS RP	CCTCCGCCTCCGCCTCCGCCGCCTCCGCCAATCAACAACCGACGTTT
DIM-7 UTR FP	TGCTATACGAAGTTATGGATCCGAGCTCGGGCCTTCCTGCATTATTG
DIM-7 UTR RP	ACCGCGGTGGCGGCCGCTCTAGAACTAGTACAGGAGCAATTATACCA
DIM-7-NotI FP	CGGCTGCTAGGAGGGGCGCGAA
DIM-7-Xba FP	CTTACAACAAAGCGACGTATATCG
DIM-7-PacI RP	CCCTTAATTAAATCAACAACCGACGTTTGAT
delta NT domain P1	AAGGGTGTTAGGTTCATATCCGATCGA
delta NT domain P2	TACTATG GCATC ATGGGTCCGTACG AG
delta ZF P1	GGTCCCGTTAGGACCGGCCGGTG
delta ZF P2	GAGGAGCAGGCGCAGAAGATCAA
C579G P1	TCCTAACGGGACCTGGACGGGCGATCAGGAAGGGTGCAACTAC
C579G P2	GTAGTTGCACCCTTCCTGATCGCCCGTCCAGGTCCCGTTAGGA
hH4 FP	AACCACCGAAACCGTAGAGGGTAC
hH4 RP	ATCGCCGACACCGTGTGTTGTAAC
8A6 FP	GGATGGCGGATCCTCAAAAATA
8A6 RP	TAACCGCCGCTTTTTAAAATTAGGA
8G3 FP	CGTAGAGAAGGGAAGTAGTAGAAGG
8G3 RP	GCACAATACGAAGTCACTTTTCACC
9E1 FP	TAAACGCAGCCGCCCAGCCC
9E1 RP	TTTAATCTACTAGGCAATACGATATACG
his-3 FP	CTATAGGGCGAATTGGGTACCGGGCCCCCCTTGCCATCTCCACCATCCTCT
his-3 RP	GGATCCCCCGGGCTGCAGGAATTCGGGCGTGCACGGCTATGGGGTC
his3flank fp	CTTAA G G G G CCAAG CTACCCCGTCAAAT
his3flank rp	TCACTAAAGGGAACAAAAGCTGGAGCTCCAGGATTCGCTCACTCGGTGCCGC
C_dam_fp	GGCGGAGGCGGCGGAGGCGGAGGCGGAGGCAAGAAAAATCGCGCTTTTTTGA
C_d a m_hisflank_rp	ATTTGACGGGGTAGCTTGGCCCTTATTTTTTCGCGGGTGAAAC
dim5_his3_fp	GACCCCATAGCCGTGCACGCCCTGAATGCAGGCCCTGGCTTTCGCGGATAAGGTTG
dim5_gly_rp	GCCTCCGCCTCCGCCTCCGCCGCCTCCGCCCCTCCGCCTCCGCCTCCGCCG