Supplementary Materials for

Epigenetic maintenance of DNA methylation after evolutionary loss of the *de novo* methyltransferase

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Materials and Methods

Yeast growth and manipulations

Yeast manipulation and protocols were performed as described in(*19*). Tagging, deletions and insertions of genes were obtained through homologous recombination by transforming about 10 µg of either a PCR product or a PmeI-digested plasmids bearing the sequence to be transformed. The *RI-DMT5* strain used for WGBS was produced by deleting the 5' portion of the *DMT5* gene and then restoring it by homologous replacement. To be certain that no residual DNA methylation was present in *dmt5*Δ prior to introduction of the targeting construct that restores the gene, the *dmt5*Δ strain was first propagated in YPAD for at least 90 generations.

To express DnmtX in an *RI-DMT5* strain, genomic DNA from *C. pinus, K. mangroviensis* and *C. bestiolae* was used as a template to amplify the genes putative DnmtXs (*K. mangroviensis* I203_05465, *C. pinus* I302_00877, *C. bestiolae* I206_02865). DnmtX was cloned downstream a *GAL7* promoter and the entire construct was then inserted at the *URA5* locus. Upon transformation, cells were selected in YPAD+nourseothrycin and then propagated for two sequential single-colony streaks in presence of galactose to induce DnmtX.

Other strains generated by transformation were colony-purified, patched, verified, and frozen. Liquid cultures were inoculated directly from the frozen stock.

The list of strains used in this study can be found on Table S1.

Crosses

Crosses were carried out using strains of the KN99 background. Cells of different mating types were grown overnight in YPAD, spotted on mating plates (4%Agar, 10% 10X Murashige and Skoog basal medium, 0.1mg/ml myo-inositol) and kept in the dark for 1 to 2 weeks. Spores were collected with a toothpick and resuspended in 500 µl of water. Dilutions of this suspension were then spread on selective media.

DNA extraction and Southern analysis

DNA from *C. neoformans, C. pinus, K. mangroviensis* and *C. bestiolae* was extracted as previously described (*19*) with some modifications. In brief, 100OD of yeast (100ml, OD1) were harvested, frozen in liquid nitrogen, resuspended in 5ml CTAB buffer (100mM Tris pH 7.6, 1M NaCl, 10mM EDTA, 1% Cetyltrimethyl ammonium bromide, 1% ß-mercaptoethanol) and incubated at 65ºC for at least 1h. 3ml of chloroform were added to the lysate, mixed and spun for 5min at 3000g. The aqueous phase was then precipitated by adding the same volume of isopropanol. The dried pellet was resuspended in 300 µl TE containing 1µg RNAse A (Thermo Scientific) and incubated 30min at 37ºC followed by addition of 3µl Proteinase K (20mg/ml) and incubation at 50ºC for 1h. Phenol-chloroform and chloroform extraction was carried out and the DNA is finally precipitated by addition of 1/10volume NaOAc and three volumes of ethanol. For Southern analysis, 10µg were then digested with the meCpG sensitive enzyme HpyCH4IV (NEB), separated by electrophoresis on a 1% agarose gel and transferred to nylon membrane (Hybond NX, Amersham) by capillary action. DNA was crosslinked to the membrane using UV light. PCR products were used as template to incorporate radioactive ^{32}P-dCTP (Perkin Elmer) using the High Prime kit (Roche) according to manufacture's protocol. Primer sequences for the probes are listed in Table S2.

Stress conditions
To assess if specific stress conditions induce *de novo* DNA methylation activity, wild-type and RI-Dnmt5 cells were grown in the presence of stressors. 2.3M NaCl at 30°C for 3h, 140mM/2.3mM $NaNO_2$/Succinic acid at 30°C for 3h, 0.14% H_2O_2 at 30°C for 3h, 0.9% SDS at 30°C for 3h, 1.9mg/ml Caffeine at 37°C for 20h, DMEM at 37°C, 5% CO_2, for 24h . The concentrations of stressors used represent the maximum concentration that allows growth in the conditions tested.

Protein extraction and western analysis
Two millilitres of culture at OD600=1 were collected by centrifugation and frozen in liquid nitrogen, resuspended in 10%TCA and incubated on ice for 10min. Cells were washed once with acetone and air-dried for 10min. The pellet was then resuspended in 150µl 2x Laemmli buffer adjusted with 80µl Tris-HCl (pH 8.0) and bead-beated 2x90s. The lysate was boiled for 5min and centrifuged to remove residual cells. Western analysis was performed using anti-FLAG antibody (1:3000- F3165, sigma), anti-HA (1:10000- 26183, Thermo Fisher) or anti-H3 antibody (1:1000- PA5-16183, Thermo Fisher) diluted with 5% milk in TBS-T (10 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20) for 1h followed by 2x10min washes in TBS-T. The membrane was then incubated with antibody anti-rabbit or anti-mouse conjugated to HRP (BIO-RAD) (1:8000 in 5% milk+TBS-T) for 45min followed by 2x10min washes in TBS-T. The membrane was then incubated in SuperSignal West pico luminol (Thermo Scientific) and visualised on film.

Bisulfite conversion and library construction
Bisulfite library construction was performed as previously described(*20*). Briefly, 100 OD units of yeast were harvested, lyophilised overnight and the DNA was extracted using DNeasy Plant Mini Kit (Qiagen). 5ng of unmethylated
l-DNA (Promega) was added to 2µg of yeast DNA and fragmented using a Bioruptor Pico instrument (Diagenode- 13 cycles, 30s/30s On/Off). The DNA was end-repaired and ligated to methylated adapters. Two rounds of bisulfite conversion of the adapter-ligated DNA were performed using EZ-DNA methylation Gold Kit (D5005- Zymo Research), and used as template for three separate PCR reactions (eight cycles of amplification) as described in (*20*). DNA obtained from the three PCR reactions were then combined and analysed for size on a High Sensitivity DNA chip (Agilent Technologies). Reads from the bisulfite-treated library were analyzed with BS-Seeker2 v2.0.3 (*21*) using bowtie1 as the aligner. bs_seeker2-call_methylation.py was run using the option '-x' which removes reads that are considered not fully converted by bisulfite treatment. To quantify CG methylation levels globally, data were further filtered using a custom Python script for signals at CG dinucleotide that displayed a fractional methylation of 0.5 or greater on both strands.

Chromatin Immunoprecipitation
ChIP and library preparation were performed as previously described (*22*) with the following modifications. Cells were lysed using a bead beater (8 cycles x 90s) and lysate

was clarified by centrifugation 10min at 6800g. The chromatin pellet was sonicated in a Bioruptor Pico instrument (Diagenode- 25 cycles, 30s/30s On/Off) and spun for 20min at 20000g. The cleared lysate was incubated overnight with 6 µg anti-histone H3K9me2 (ab1220, Abcam) or 4µl anti-FLAG antibodies (F3165, sigma) with the addition of 30µl of protein A (for anti-H3K9me) or protein G (for anti-FLAG) Dynabeads (Thermo Fisher). Library preparation and bioinformatics analysis were performed as described in (*22*).

Methylated DNA immunoprecipitation (MeDIP)

The initial steps are the same as described for the bisulfite library protocol (*20*) with the exception that for MeDIP, unmethylated adapters were ligated to repaired, dA-tailed DNA. Upon ligation and purification, DNA was boiled for 10min and kept on ice for 5min. Ice cold binding buffer (10mM sodium phosphate buffer, pH 7, 140mM NaCl 0.05%Triton X-100) was added to the DNA together with 1µl of anti-5-methylcytosine antibody (A-1014-050, Epigentek) and incubated on a nutator overnight at 4ºC. 20µl Protein G Dynabeads (Thermo Fisher) were added and incubated on a nutator for further 2h at 4ºC. Beads were washed 10x5min in binding buffer and the DNA was eluted by incubating the beads with Elution Buffer (TE, 0.25mg/ml Proteinase K, 0.25% SDS) for 2h at 55ºC. The DNA was purified with NusleoSpin PCR clean-up columns (Macherey-Nagel) using NTB buffer (Macherey-Nagel) and PCR amplified as for ChIP. Bioinformatics analyses were performed as for ChIP-seq.

Recombinant Protein Expression and Purification

A codon-optimized DNA sequence encoding *C. neoformans* Dnmt5 (residues 1-150) was cloned into pETARA or pMAL vectors and used to transform *E. coli* strain BL21(DE3) (*23, 24*). Transformed cells were grown to OD_{600} = 0.8 in 2x YT medium, then induced with 1 mM IPTG overnight at 18˚C. Recombinant GST-Dnmt5(1-150)-6xHis was purified with Ni-NTA agarose resin (Qiagen), measured by A_{280} (ε = 66,030 cm^{-1} M^{-1}), and used for histone peptide array binding assays. Recombinant MBP-Dnmt5(1-150)-6xHis was purified with Ni-NTA agarose resin (Qiagen), measured by A_{280} (ε = 89,590 cm^{-1} M^{-1}), and used for fluorescence polarization binding assays.

A codon-optimized DNA sequence encoding full-length *C. neoformans* Uhrf1 was cloned into pBH4 and expressed in *E. coli* as above. Recombinant 6xHis-Uhrf1 was purified with Ni-NTA agarose resin (Qiagen), measured by A_{280} (ε = 40,920 cm^{-1} M^{-1}), and used for electrophoretic mobility shift assays.

Full-length cDNA encoding Swi6 was cloned from *C. neoformans* and expressed in *E. coli* as above using the pBH4 vector. Recombinant 6xHis-Swi6 was purified with Ni-NTA agarose resin (Qiagen), measured by A_{280} (ε = 45,330 cm^{-1} M^{-1}), and used for fluorescence polarization binding assays.

For expression in *S. cerevisiae*, full-length cDNA encoding Dnmt5 was cloned from *C. neoformans*, inserted into the 83ν vector(*25*), and used to transform the *S. cerevisiae* strain JEL1(*26*). Starter cultures were grown overnight in SC -ura medium (2% glucose), then used to inoculate 2 L cultures of YPGL medium (1x YEP, 1.7% lactic acid, 3% glycerol, 0.12% glucose, 0.15 mM adenine) to a starting OD_{600} of 0.03. After growth at 30˚C to an OD_{600} of 1.0, expression was induced by addition of 2% galactose. After 6 hr of continued growth at 30˚C, cells were harvested, washed once in TBS (50

mM Tris-Cl pH 7.6, 150 mM NaCl), and snap frozen. Frozen cells were lysed in a ball mill (6x 3 min at 15 Hz), resuspended in Ni-NTA lysis buffer (50 mM NaH_2PO_4 pH 8, 300 mM NaCl, 10% glycerol, 10 mM imidazole, 2 mM β-mercaptoethanol, 0.02% NP-40, 1x CPI), and centrifuged 20,000 x *g* for 30 min at 4˚C. Lysate was bound to Ni-NTA resin in batch format for 2 hr at 4˚C, after which resin was washed in column format with five bed volumes Ni-NTA buffer followed by ten bed volumes Ni-NTA wash buffer (identical to Ni-NTA lysis buffer except 20 mM imidazole). Finally, bound protein was eluted with four bed volumes of elution buffer (identical to Ni-NTA lysis buffer except 300 mM imidazole and no detergent). Protein was dialyzed against storage buffer and applied to a HiTrap Q HP anion exchange column (GE Life sciences) pre-equilibrated in buffer (50 mM HEPES-KOH pH 7.9, 150 mM KCl, 10% glycerol, 2 mM β-mercaptoethanol). Fractions were collected across a 150-1000 mM KCl gradient, and those containing Dnmt5 were pooled, dialyzed against storage buffer, and frozen.

Histone Peptide Array Binding Assay

Modified histone peptide arrays (Active Motif) were blocked with 5% dried milk in TBS-T (10 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20) overnight at 4˚C. Each slide was subsequently washed in TBS-T (4x5 min) and binding buffer (2x5min). Binding buffer consisted of 50 mM HEPES-KOH pH 7.9, 200 mM NaCl. The slide was incubated with 0.5 μM GST-Dnmt5(1-150) in binding buffer for 4 hr at 23˚C. It was then washed with TBS-T (4x 5 min). Next, the slide was incubated with anti-GST antibody (Sigma G7781; 1:13,000 dilution) in 5% dried milk in TBS-T for 1hr at 23˚C. After washing in TBS-T (4x 5 min), the slide was incubated with HRP-conjugated goat anti-rabbit secondary antibody (sc-2004, Santa Cruz Biotechnology; 1:5,000 dilution) in 5% milk in TBS-T for 1hr at 23˚C. The slide was washed in TBS-T (4x 5 min) and visualized using SuperSignal West Pico chemiluminescent substrate (Pierce) and a ChemiDoc MP System (BioRad). Signal intensity normalization was performed using Array Analyze Software (Active Motif).

Fluorescence Polarization Binding Assay

Peptides were synthesized by GenScript (Piscataway, NJ) or Peptide 2.0 (Chantilly, VA) to >95% purity. For study of H3K9 methylation, unlabeled peptides corresponded to residues 1-15 of *C. neoformans* histone H3. For study of H3K27 methylation, unlabeled peptides corresponded to residues 23-34 of *C. neoformans* histone H3 followed by a cysteine residue. Peptide concentrations were determined by A_{205} or, in the case of fluorescein-conjugated peptides, A_{495} (ε = 80,000 M^{-1} cm^{-1}).

For direct measurement of peptide binding, increasing concentrations of MBP-Dnmt5(1-150) were incubated with 10 nM labeled peptide in a solution of 20 mM HEPES pH 7.9, 120 mM KCl, 0.8 mM DTT, and 0.01% NP-40. Peptide fluorescence polarization was measured using a Spectramax M5e plate reader (Molecular Devices) and non-stick 384-well plates (Corning 3820). For competition assays, unlabeled competitor peptides were added at increasing concentration in the setting of 5 μM MBP-Dnmt5(1-150) or 30 μM 6xHis-Swi6 and 10 nM fluorescein-conjugated H3K9me3 peptide. Dissociation constants were calculated using a competition binding equation in Prism (GraphPad Software)(*27*). $FP_{obs} = \frac{K_i*(FP_{max}*[Dnmt5]+FP_{min}*K_d)+FP_{min}*K_d*[I]}{K_i*(K_d+[Dnmt5])+K_d*[I]}$ Dissociation

constants for H3K9me3 peptide were comparable when measured directly (1.3 μM) or as a competitor (1.5 μM).

Gel Mobility Shift Assay

Primer sequences are listed in Table 2 and were annealed to generate unmethylated, hemimethylated, or symmetrically methylated 20 bp dsDNA substrates. The substrates were then radiolabeled using the KinaseMax kit (Ambion) and ATPγ-^{32}P (Perkin Elmer), after which they were purified using a G-25 illustra microspin column (GE Life Sciences). For direct measurement of DNA binding, recombinant 6xHis-Uhrf1 was incubated with 0.2 nM labeled DNA probe in a 10 μl solution of 15 mM HEPES-KOH pH 7.9, 7.5% glycerol, 75 mM KCl, 0.075% NP-40, 0.05 μg/ul poly-dI-dC (Sigma), 0.5 μg/ul BSA, 1 mM DTT, and 5 mM $MgCl_2$. After 30 min equilibration at 23˚C, samples were resolved in polyacrylamide gels (4.5% acrylamide:bis 29:1 (Bio-Rad), 1% glycerol, 0.25X TBE) at 4˚C. Gels were subsequently dried and imaged using a storage phosphor screen (Amersham) and a Typhoon 9400 imager (Amersham). Densitometry was performed using ImageJ. For competitive binding assays, conditions were as above except Uhrf1 protein was kept constant at 150 nM in the presence of 0.2 nM labeled hemimethylated probe and excess amounts of unlabeled DNA oligonucleotides.

DNA Methyltransferase Assay

DNA oligonucleotides were synthesized and annealed to generate 20 or 60 bp dsDNA substrates (Table 2). DNA methylation was monitored in multiple turnover conditions by incubating 30 nM Dnmt5 in a solution of 50 mM Tris pH 8, 25 mM NaCl, 10% glycerol, and 2 mM DTT, in the presence of 5 μM DNA substrate. When indicated, ATP and $MgCl_2$ were supplemented at 1 mM, histone tail peptides corresponding to histone H3 residues 1-15 were added at 5 μM, Uhrf1 was added at 4 μM, and Swi6 was added at 4 μM. Reactions were initiated by the addition of 4 μM ^{3}H-SAM (Perkin Elmer) and carried out at 23˚C. Reaction aliquots were removed at indicated time points and quenched in a solution of 10 mM SAM in 10 mM H_2SO_4. The quenched solution was pipetted onto DE81 filter paper and allowed to dry for 10 min. Filter papers were subsequently washed three times in 200 mM ammonium bicarbonate (5 min each), then once with water (5 min). Filter papers were then rinsed twice in ethanol, after which they were dried for 20 min. Filters were added to scintillation fluid (Bio-Safe NA, Research Products International Corp.), and bound ^{3}H was detected in an LS 6500 scintillation counter (Perkin Elmer). Background signal was assessed using reactions that lacked Dnmt5 enzyme. Detectable signal was defined as reactions that exhibited cpm greater than 2-fold above background at each measured time point. Background signal was typically 50-100 cpm and signal for productive reactions ranged from ~1000 to ~100,000 cpm depending on conditions and time point. For productive reactions, separate experiments were performed to confirm that the DNA substrate was at saturating concentration. For reactions in which methylation was detected, rates were calculated over the first 15-20 min where reaction progress was linear and less than 10% of available hemimethylated sites had been acted upon. These initial rate values were divided by Dnmt5 concentration to obtain k_{obs}. DNA substrate was confirmed to be present at saturating concentration for these experiments. Serial dilutions confirmed that ^{3}H detection using DE81 was linear to the level of background signal.

Phylogenetic methods

For phylogenetic analysis, orthologs were identified across sequenced Tremellales (Cuomo, manuscript in prep) based on BLASTP pairwise matches with expect< 1e-5 using ORTHOMCL v1.4.(*28*) A phylogeny was estimated from 4080 single copy genes as follows. Individual proteins were aligned using MUSCLE (*29*) and the individual alignments were concatenated and poorly aligning regions removed with trimal.(*30*) This sequence was input to RAxML (*31*) version 7.7.8 (raxmlHPC-PTHREADS-SSE3) and a phylogeny estimated in rapid bootstrapping mode with model PROTCATWAG and 1,000 bootstrap replicates.

Figure S1- Catania et al.

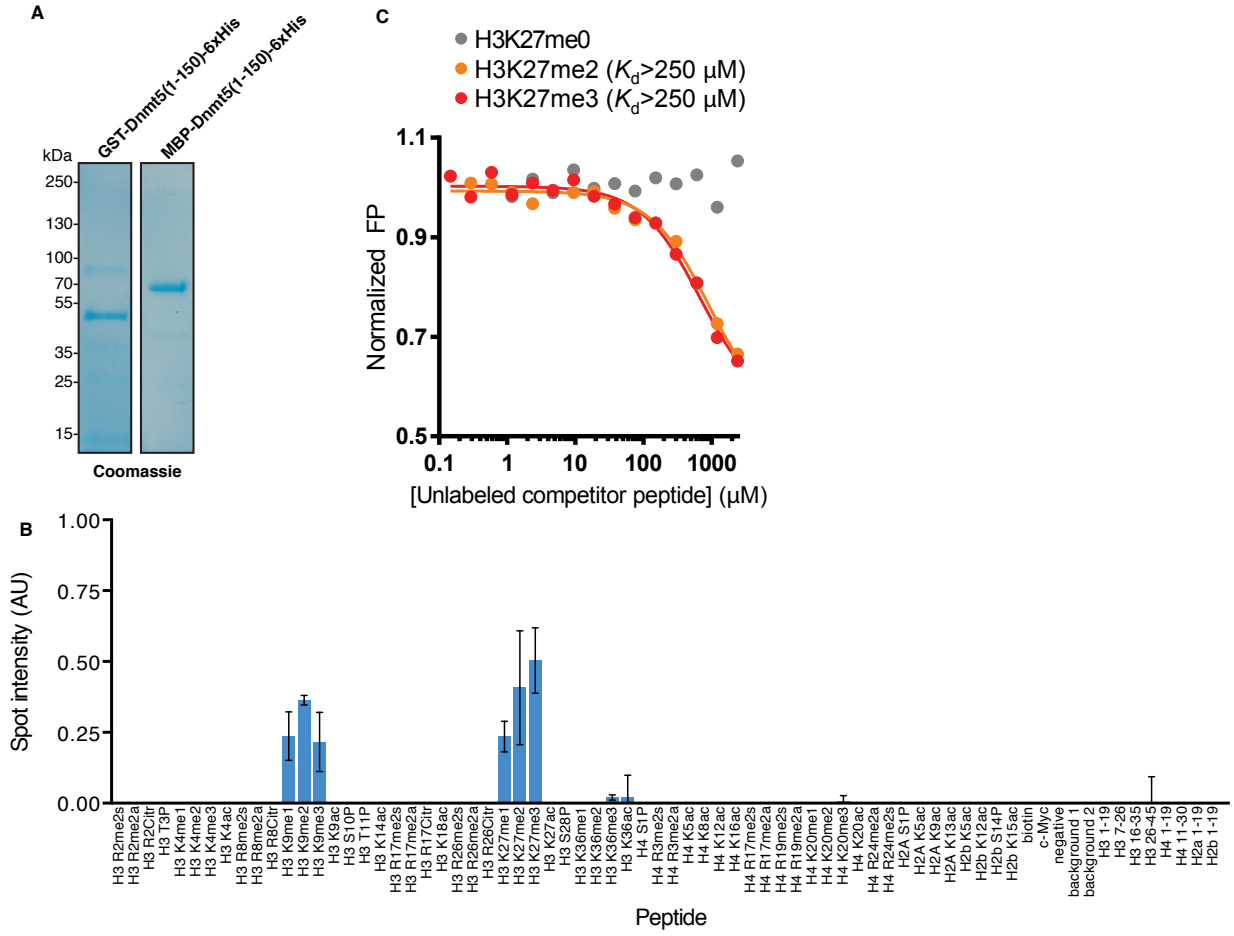

Fig. S1. The chromodomain of Dnmt5 binds to H3K9me peptides

(**A**) Coomassie Brilliant Blue staining of GST-Dnmt5(1-150) and MBP-Dnmt5(1-150) after affinity purification from lysates of *E. coli* and resolution by SDS-PAGE. This truncation contains the Dnmt5 chromodomain.

(**B**) Dnmt5(1-150)-GST binding to a peptide microarray of human-derived histone sequences and modifications. Intensity represents background-subtracted signal at peptide array spots exhibiting the modifications indicated below the graph. Data are the average from two peptide arrays; error bars represent SE.

(**C**) Binding of MBP-Dnmt5(1-150) to H3K27 peptides, as assessed by a fluorescence polarization binding competition assay. Dnmt5(1-150) was bound to a fluorescently-labeled H3K9me3 peptide in the presence of increasing concentrations of unlabeled H3K27me0/2/3 peptides corresponding to a region of *C. neoformans* H3. Polarization was normalized to that observed in the absence of competitor peptide; K_d calculated from 2-3 replicates.

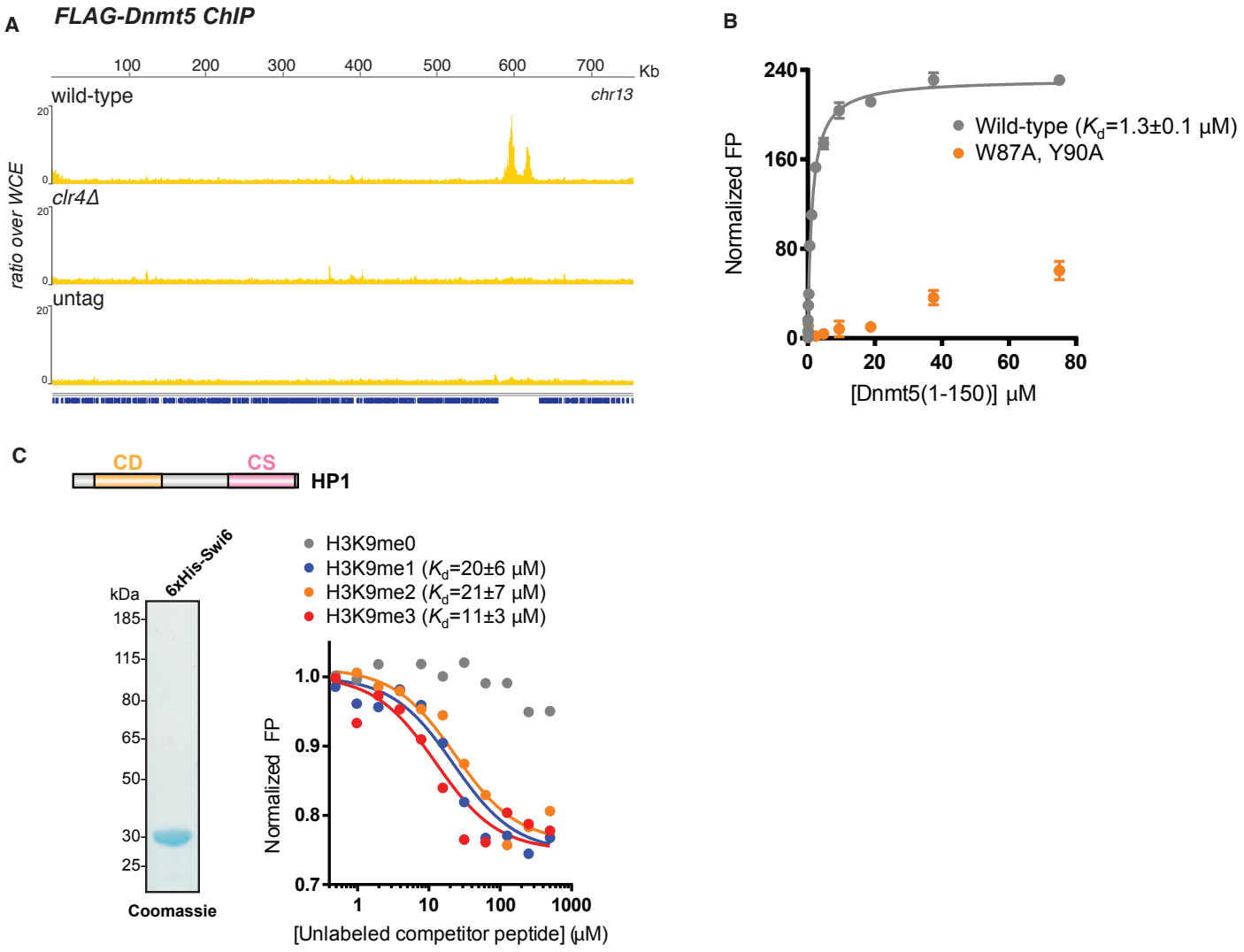

Fig. S2. Interactions of Dnmt5 and heterochromatin

(**A**) ChIP-seq analysis of FLAG-Dnmt5 in wild-type and *clr4*Δ backgrounds compared to untagged strain. Shown are the data for chromosome 13.
(**B**) Effect of chromodomain aromatic cage mutations on binding of MBP-Dnmt5(1-150) to H3K9 peptides. Wild-type or *W87AY90A* mutant MBP-Dnmt5(1-150) was incubated at increasing concentrations with H3K9me3 peptide. Polarization was normalized such that a value of 1 was equivalent to the polarization in the absence of MBP-Dnmt5(1-150) protein. (**C**) Coomassie staining of 6xHis-Swi6/HP1 after purification from *E. coli* and resolution by PAGE. Left: chromodomain and chromoshadow domains of full-length Swi6 are indicated. Right: binding of Swi6 to H3K9 peptides, as assessed by fluorescence polarization binding competition assay. Swi6 was bound to labeled H3K9me3 peptide in the presence of increasing concentrations of unlabeled H3K9me0/1/2/3 peptides. Polarization was normalized to that observed in the absence of competitor peptide; K_d represents average ± 95% CI of 2-3 replicates

Figure S3. Catania et al.

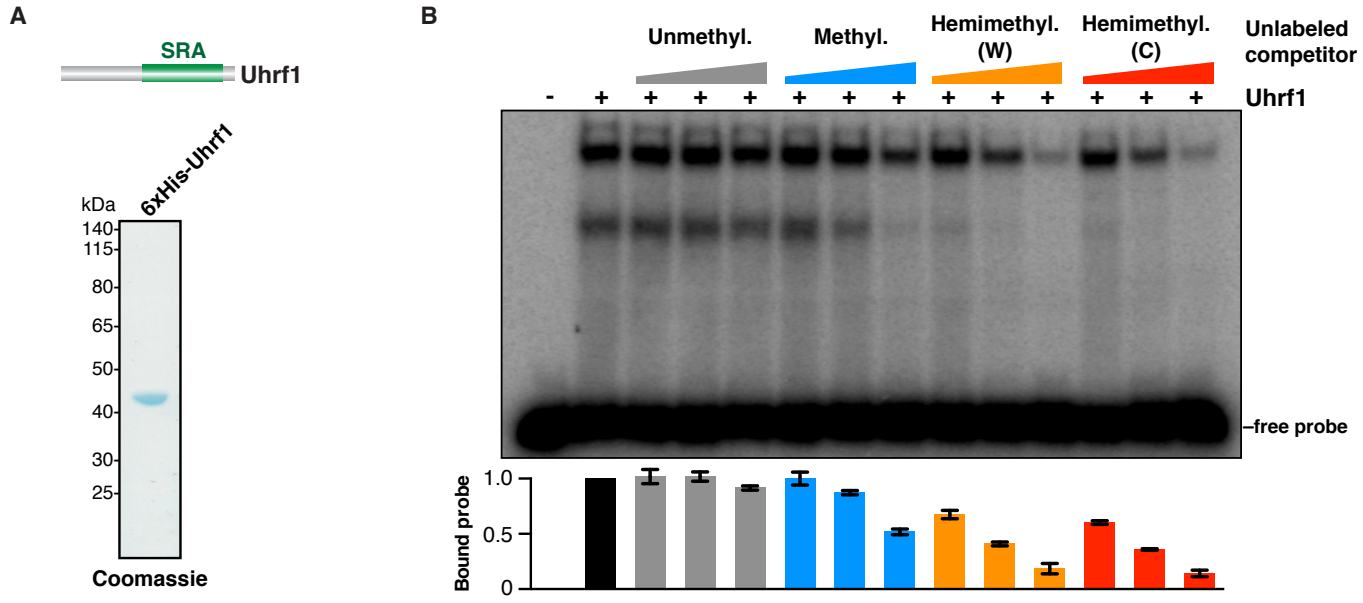

Fig. S3. Uhrf1 selectively binds DNA harboring hemimethylated CG dinucleotides

(**A**) Top: Domain structure of *C. neoformans* Uhrf1 ortholog. Bottom: Coomassie Brilliant Blue staining of Uhrf1-6xHis after purification from *E. coli* and resolution by SDS-PAGE. Predicted protein domains of full-length Uhrf1 are indicated.
(**B**) EMSA assessing competition between labeled hemimethylated DNA probe and excess concentrations unlabeled DNA (0.2, 1.0, 5.0 µM) of indicated methylation state. Where indicated, Uhrf1 protein was added at 150 nM. Graph indicates fraction of probe bound relative to condition in which no cold competitor was added; n=2, error bars represent SD.

Figure S4. Catania et al.

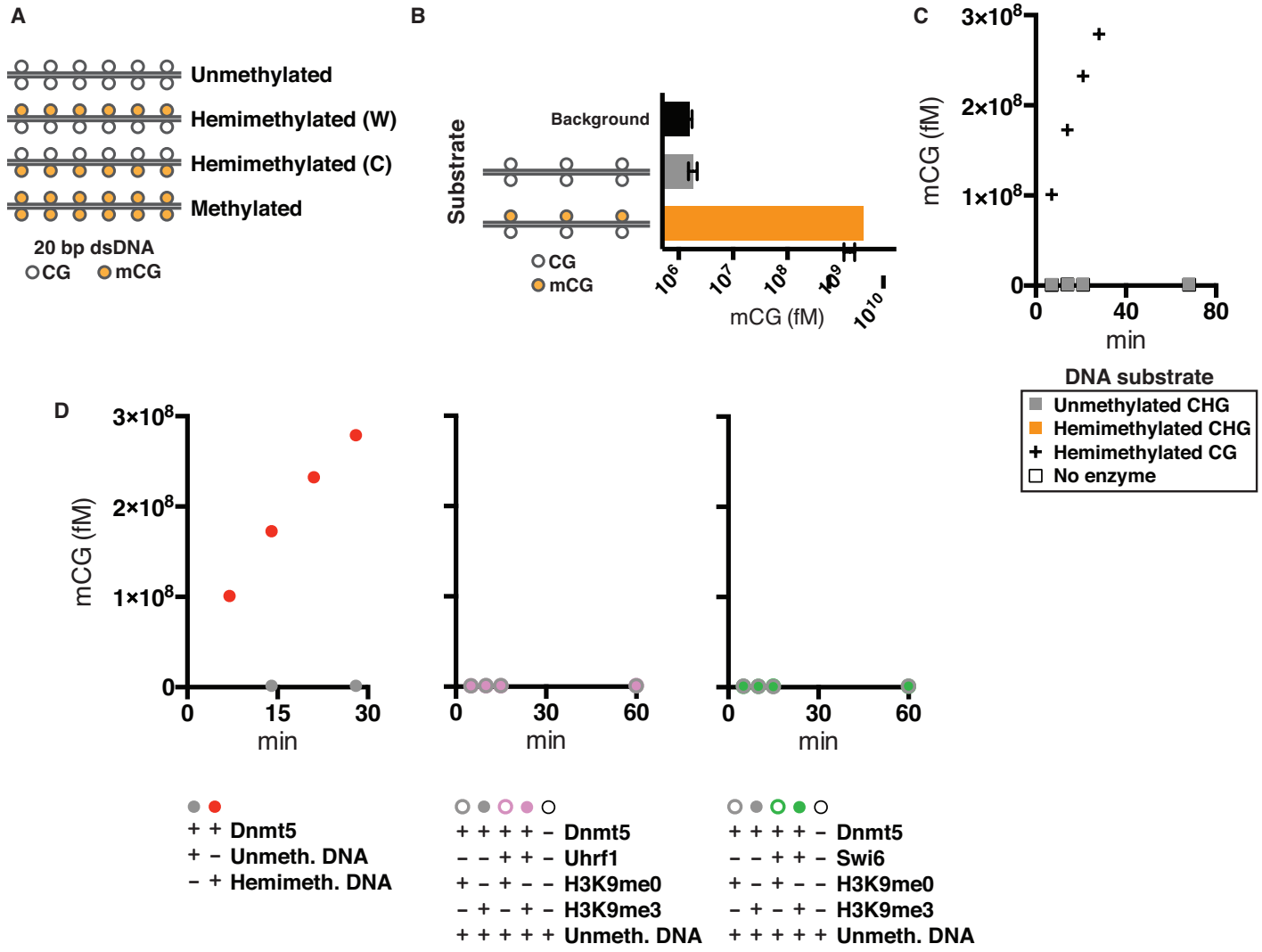

Fig. S4. Hemymethylated DNA but not unmethylated DNA is a substrate for purified Dnmt5.

(**A**) Double stranded DNA substrates used in methyltransferase experiments. Each 20 bp substrate contains 6 CG sites that are either unmethylated, hemimethylated, or symmetrically methylated.
(**B**) End point measurement of DNA methylation activities on methylated versus unmethylated substrates after a 6 hr reaction of 100 nM Dnmt5 and 5 μM DNA substrate. Background: no enzyme control. Error bars represent SD; n = 3.
(**C**) DNA methylation kinetics using 30 nM Dnmt5 and 5 μM DNA substrates containing either CHG or CG motifs in an unmethylated or hemimethylated state.
(**D**). Left: control DNA methylation reaction in which 30 nM Dnmt5 was incubated with 1 mM ATP and 5 μM of the unmethylated or hemimethylated DNA substrates. Center and right: DNA methylation reactions in which 30 nM Dnmt5 was incubated with 1 mM ATP and 5 μM unmethylated DNA in the presence or absence of 5 μM H3K9me0/3 peptide, 4 μM Uhrf1, and/or Swi6/HP1.

Figure S5 - Catania et al.

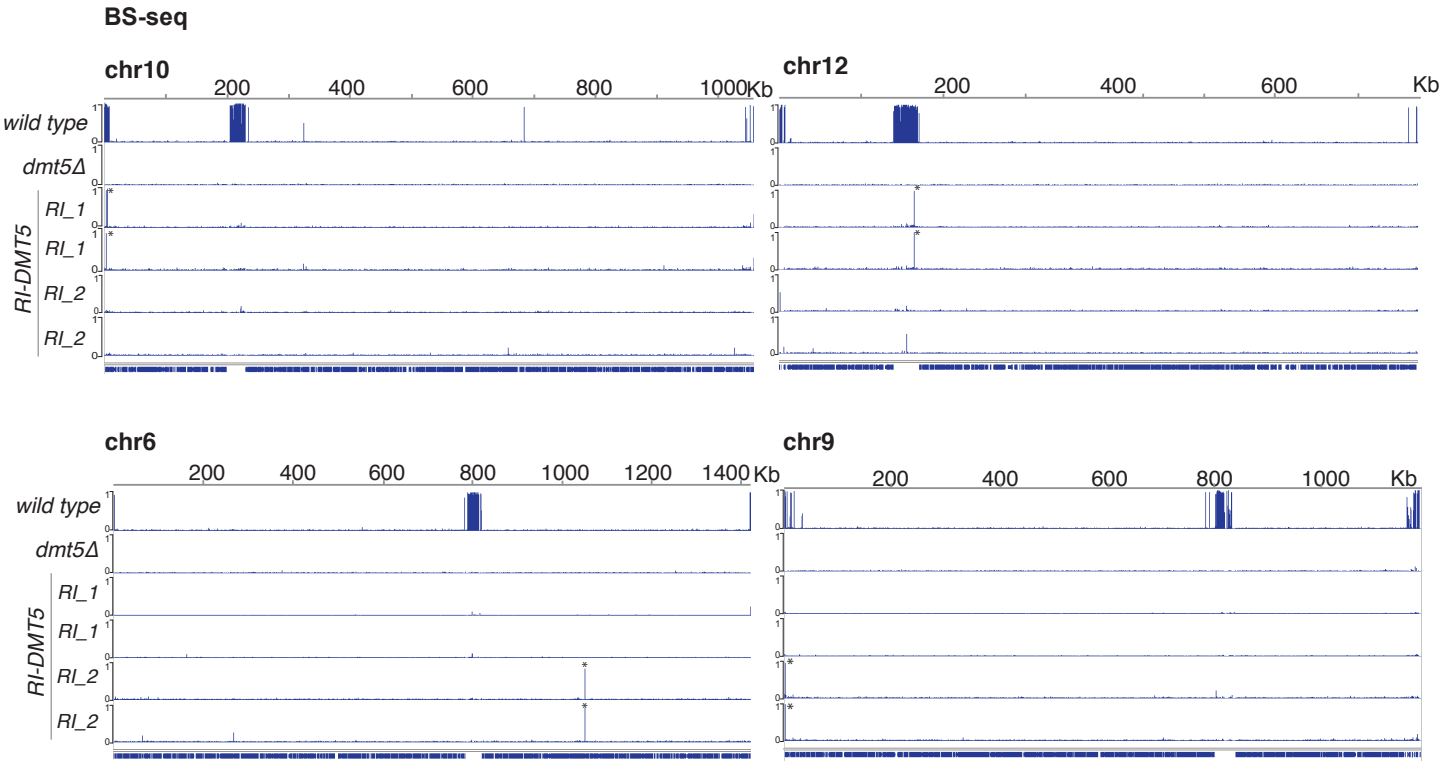

Fig. S5. WGBS of two independently-derived *RI-DMT5* strains.

DNA from two independently derived strains (RI_1 and RI_2) were bisulfite treated in technical duplicate. Shown are the peaks present in both technical duplicates (marked with asterisks).

Figure S6. Catania et al.

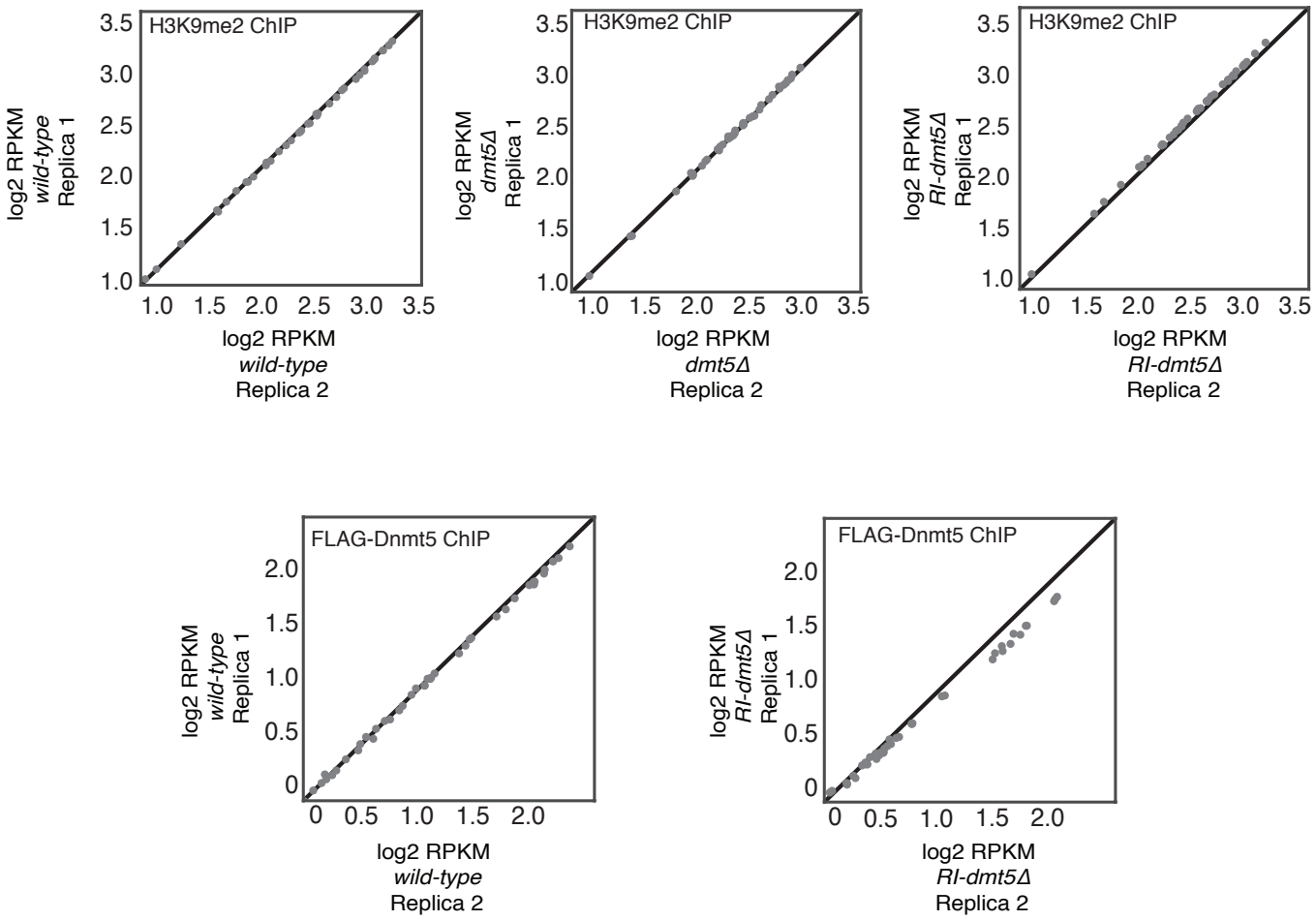

Fig. S6. Reproducibility of biological replicates for ChIP analysis.

Scatter plot analysis of anti-H3K9me2 and anti-FLAG ChIP. Points represent the signals for each centromere and telomere (RPKM). Each sample was compared against a biological replicate.

Figure S7. Catania et al.

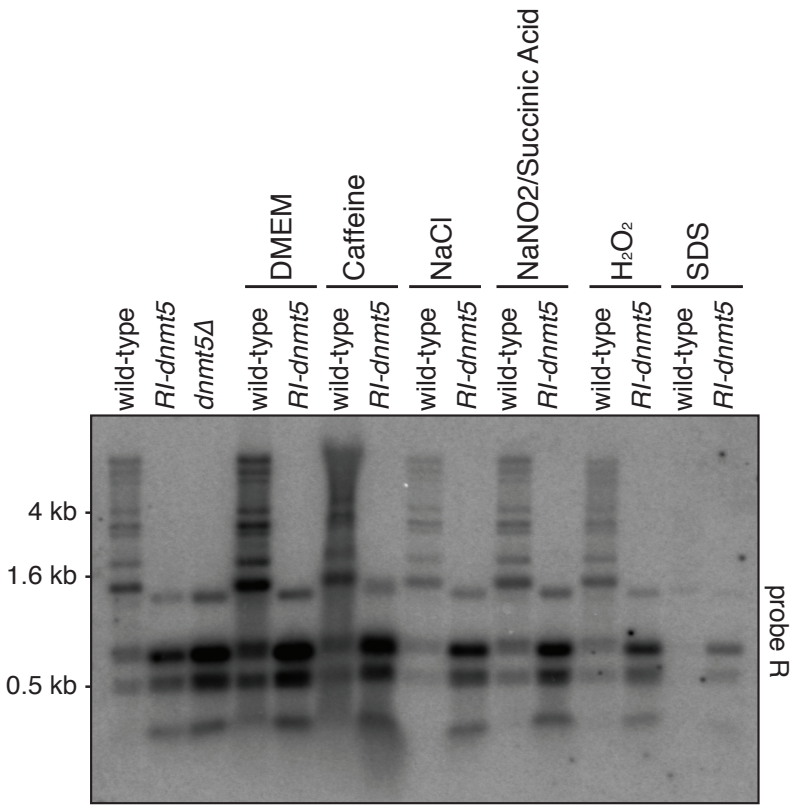

Fig. S7. Stress conditions do not induce *de novo* activity in Dnmt5

To assess if specific stress conditions induce *de novo* DNA methylation activity, wild-type and RI-Dnmt5 cells were grown in the presence of stressors The genomic DNA was extracted, digested with the CG methylation sensitive endonuclease HpyCHIV and analysed by Southern hybridization using a probe corresponding to a repetitive centromeric sequence (probe R).

Figure S8. Catania et al.

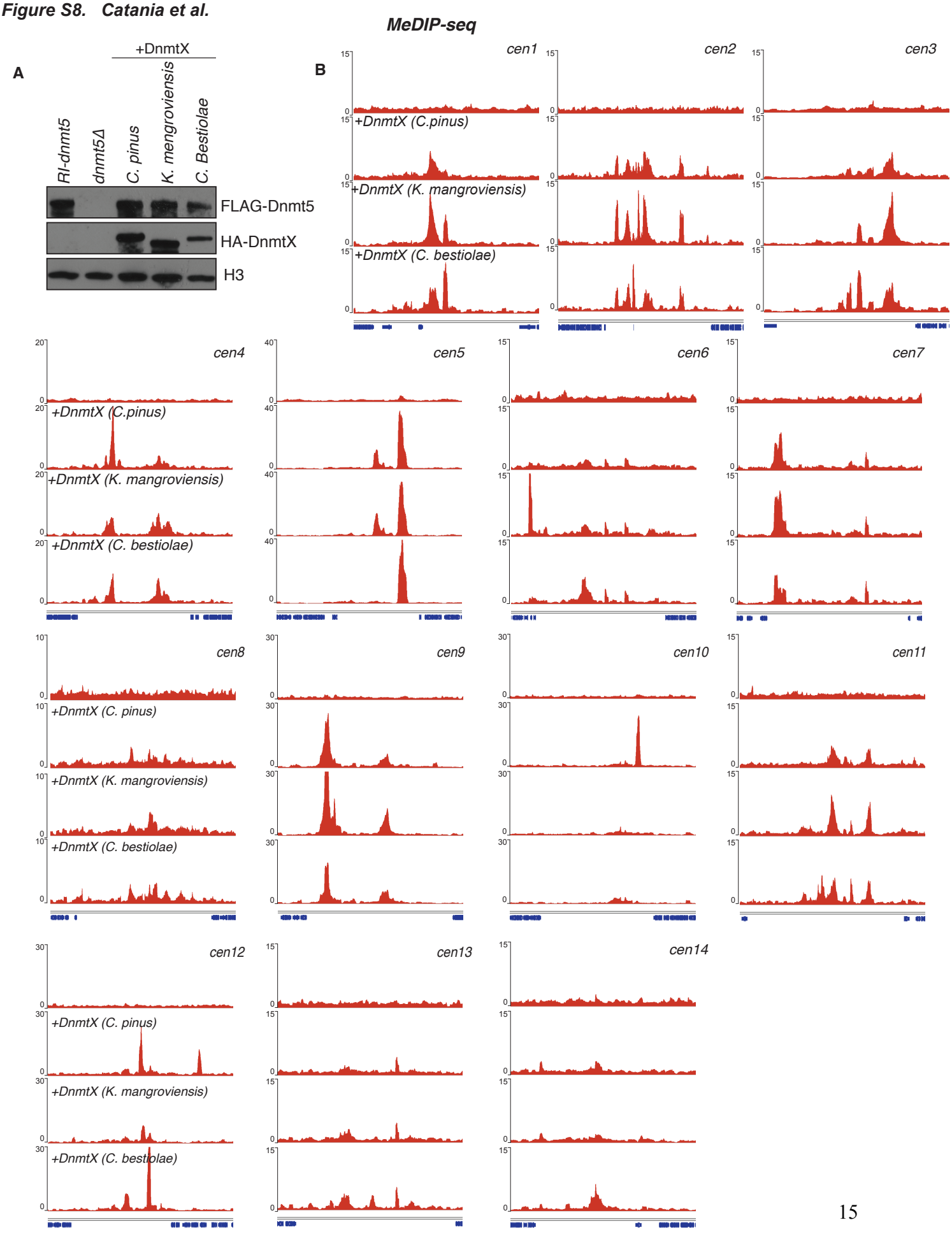

Fig. S8. DnmtX acts as a *de novo* DNA methyltransferase in cells expressing RI-Dnmt5.

(**A**) HA-tagged version of DnmtX from *C.pinus, K. mangroviensis* and *C. bestiolae* were expressed under the control of *GAL7* promoter in an *RI-DMT5* strain. Expression of the proteins was assessed by western blotting using indicated antibodies.
(**B**) MeDIP signals for the 14 centromeres from cells expressing DnmtX (+DnmtX) compared to the control without DnmtX (top). Strains were analysed after 14 days of induction of DnmtXs on YP-galactose solid media. The signals shown normalized to that of a *dmt5*Δ strain.

Table S1. List of strains used in this study

Strain number	Genotype	Parent	*Notes*	Fig.
CM229	Wild type	CM018	Wild type	1
CM1197	*CNAG_05404Δ::natR*	CM229	*clr4*Δ	1
CM1837	*CNAG_07552Δ::kanR*	CM229	*dmt5*Δ-1.5 kb from ATG	1
CM1838	*CNAG_00677Δ::natR*	CM229	*uhf1*Δ	1
CM1839	*05404Δ::natR 00677Δ::kanR*	CM1197	*clr4*Δ *uhf1*Δ	1
CM1840	*hygR-2xFLAG-CNAG_07552-W87A Y90A*	CM229	*CDmut-dmt5*	1
CM1841	*CNAG_03458Δ::kanR*	CM229	*swi6*Δ	1
CM1842	*hygR-2xFLAG-07552-W87A Y90A CNAG_03458Δ::kanR*	CM1840	*CDmut-dmt5 swi6*Δ	1
CM1843	*hygR-2xFLAG-07552*	CM229	*FLAG-DMT5*	1,3,4
CM1844	*hygR-pGAL7-2xFLAG-07552*	CM229	*pGAL-DMT5-OFF*	3A
CM1845	*hygR-pGAL7-2xFLAG-07552*	CM229	*pGAL-DMT5-ON*	3B-C
CM1846	*hygR-2xFLAG-CNAG_07552-RI*	CM1837	*RI-DMT5*	4
CM1847	*hygR-2xFLAG-CNAG_07552-RI*	KN99	*RI-DMT5*	5
CM1848	*MATa CEN13:natR*	KN99a		4E
CM1849	*MATa CEN2:kanR hygR-2xFLAG-CNAG_07552-RI*	KN99a		4E
CM1850	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1851	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1852	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E

CM1853	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1854	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1855	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1856	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1857	*CEN13::natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1858	*CEN13:natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1859	*CEN13:natR CEN2::kanR hygR-2xFLAG-07552-RI*	KN99	CM1848x1849	4E
CM1860	*MATa CEN13::natR*	KN99a		4F
CM1861	*MATa CEN2::hygR CNAG_07552Δ::kanR*	KN99a		4F
CM1862	*CEN13:natR CEN2:hygR*	KN99	CM1861x CM1860	4F
CM1863	*CEN13::natR CEN2:hygR*	KN99	CM1861x CM1860	4F
CM1864	*CEN13::natR CEN2::hygR*	KN99	CM1861x CM1860	4F
CM1865	*CEN13::natR CEN2:hygR 07552Δ::kanR*	KN99	CM1861x CM1860	4F
CM1866	*MATa CEN2::hygR*	KN99a		4G
CM1867	*MATa CEN13::natR CNAG_07552Δ::kanR*	KN99a		4G
CM1868	*CEN13::natR CEN2::hygR*	KN99	CM1866 x CM1867	4G
CM1869	*CEN13::natR CEN2::hygR*	KN99	CM1866 x CM1867	4G
CM1870	*CEN13::natR CEN2::hygR*	KN99	CM1866 x CM1867	4G
CM1871	*CEN13::natR CEN2::hygR 07553Δ::Neo*	KN99	CM1866 x CM1867	4G
CM1872	*URA5Δ::pGAL7-3xHA-I203_05465-natR hygR-2xFLAG-07552-RI*	KN99	DnmtX from *K. mengroviensis*	5
CM1873	*URA5Δ::pGAL7-3xHA-I206_02856-natR hygR-2xFLAG-07552-RI*	KN99	DnmtX from *C. pinus*	5
CM1874	*URA5Δ::pGAL7-3xHA-I302_00877-natR hygR-2xFLAG-07552-RI*	KN99	DnmtX from *C. bestiolae*	5

Table S2.
Oligonucletides used in this study

C#	Sequence (M=5mC)	Name	Strand	Assay
6153	CCCACTGGATGAAACCTCGT	Probe U/cen13	5' primer	Southern
6154	TCGGTGGGATGAGCAGAAAAAC	ProbeU/cen13	3' primer	
6155	TGGACCGGAACACCGTAGA	Probe R	5' primer	Southern
6156	ATACCGGTACGGGTGCATG	Probe R	3' primer	
6665	CACGCGACGCACGACGCGAA	Unmethylated	Watson	EMSA, DMT
6664	TTCGCGTCGTGCGTCGCGTG		Crick	
6661	CAMGMGAMGCAMGAMGMGAA	Hemimethylated (W)	Watson	EMSA, DMT
6664	TTCGCGTCGTGCGTCGCGTG		Crick	
6665	CACGCGACGCACGACGCGAA	Hemimethylated (C)	Watson	EMSA, DMT
6662	TTMGMGTMGTGMGTMGMGTG		Crick	
6661	CAMGMGAMGCAMGAMGMGAA	Methylated	Watson	EMSA, DMT
6662	TTMGMGTMGTGMGTMGMGTG		Crick	
7289	CATGGCCTAAGCCGGACTGAATGAGCAAGCTTCA GGAGAATTCTGCCGGACTGCAGATGC	A	Watson	DMT
7290	GCATCTGCAGTCCGGCAGAATTCTCCTGAAGCTT GCTCATTCAGTCCGGCTTAGGCCATG		Crick	
7287	CATGGCCTAAGCCGGACTGAATGAGCAAGCTTC MGGAGAATTCTGCCGGACTGCAGATGC	B	Watson	DMT
7288	GCATCTGCAGTCCGGCAGAATTCTCMGGAAGCTT GCTCATTCAGTCCGGCTTAGGCCATG		Crick	
7291	CATGGCCTAAGCAGGACTGAATGAGCAAGCTTC AGGAGAATTCTGCAGGACTGCAGATGC	C	Watson	DMT
7292	GCATCTGCAGTCCTGCAGAATTCTCCTGAAGCTT GCTCATTCAGTCCTGCTTAGGCCATG		Crick	
7777	CATGGCCTAAGCAGGACTGAATGAGCAAGCTTC MAGAGAATTCTGCAGGACTGCAGATGC	Hemimethylated CHG	Watson	DMT
7896	GCATCTGCAGTCCTGCAGAATTCTCTGGAAGCTT GCTCATTCAGTCCTGCTTAGGCCATG		Crick	
7817	CATGGCCTAAGCAGGACTGAATGAGCAAGCTTCC AGAGAATTCTGCAGGACTGCAGATGC	Unmethylated CHG	Watson	DMT
7896	GCATCTGCAGTCCTGCAGAATTCTCTGGAAGCTT GCTCATTCAGTCCTGCTTAGGCCATG		Crick	