SUPPLEMENTAL FIGURES

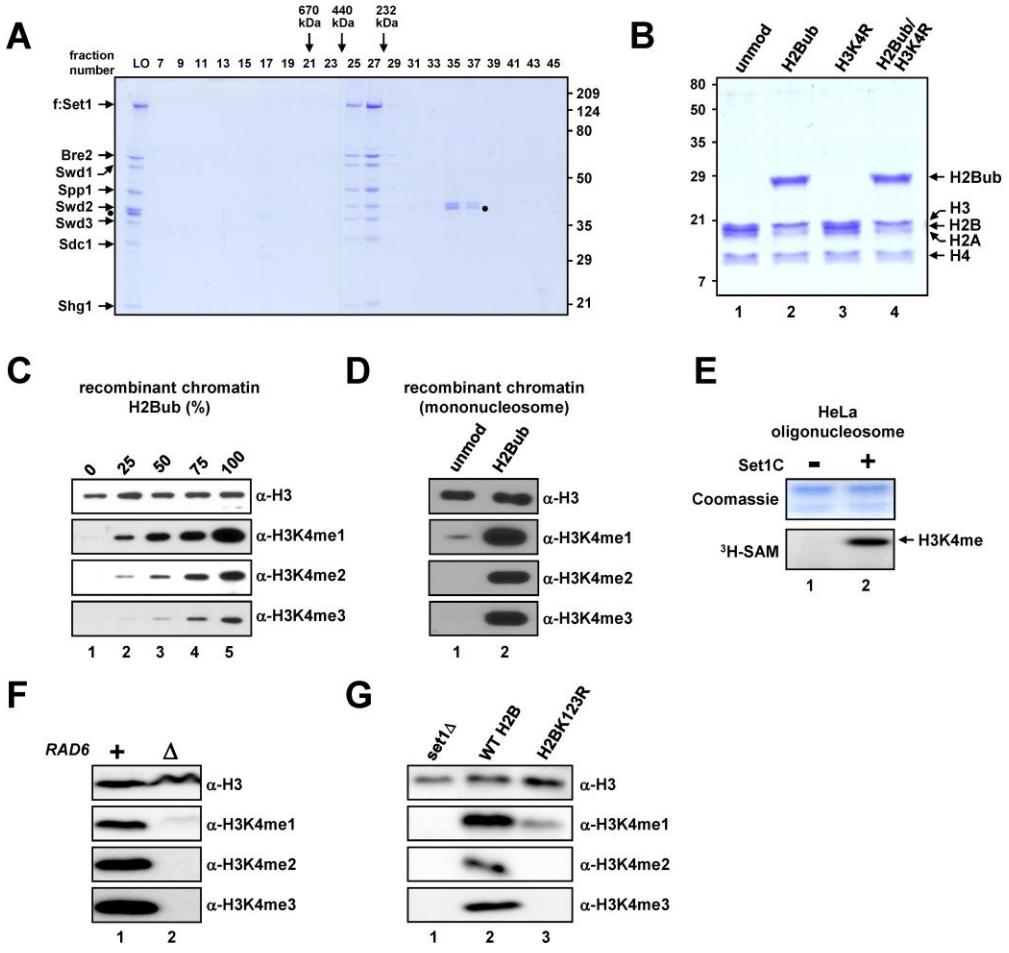

Figure S1

Figure S1. H2B Ubiquitylation-Dependent Stimulation of H3K4 Methylation by the Yeast Set1 Complex, Related to Figure 1

(A) SDS-PAGE/Coomassie Blue staining of purified ySet1C following Superose 6 gel filtration. The dot indicates an N-terminal degradation product of the FLAG-tagged Set1 that was identified by mass spectrometric analysis and is recognized by anti-FLAG antibody in this and other figures (see also Figure 1A). Note that this degradation product was separated from the intact ySet1C by gel filtration and that Swd2, while clearly evident in the intact ySet1C (fractions 25 and 27), also co-eluted with the N-terminal Set1 degradation product (fractions 35 and 37). This result suggests that Swd2 interacts directly with an N-terminal part of Set1 (see manuscript for more detail).

(B) SDS-PAGE/Coomassie Blue staining of unmodified H2B and semisynthetic H2Bub-containing histone octamers with or without lysine to arginine mutation at H3K4. Note: unmod, unmodified histones; H2Bub, lysine(K)120-ubiquitylated H2B; H3K4R, H3 containing an arginine(R) substitution at lysine(K)4.

(C-E) In vitro histone methyltransferase (HMT) assays with purified ySet1C and either chromatin arrays assembled with varying amounts of H2Bub-containing octamer relative to control octamer (C), recombinant mononucleosomes assembled with indicated histone octamers (D) or HeLa cell-derived oligonucleosomes (E) as substrates. H3 methylation status was monitored by indicated antibodies or by fluorography (in this and other figures). Note: H3K4me, lysine(K)4-methylated H3; H3K4me1, lysine(K)4-mono-methylated H3; H3K4me2, lysine(K)4-di-methylated H3; H3K4me3, lysine(K)4-tri-methylated H3.

(F and G) Dependency of all H3K4 methylation states on H2Bub in vivo. (F) Yeast whole cell extracts from wild-type (WT) and isogenic *rad6*Δ strains were subjected to immunoblot analyses with indicated antibodies. This image is a part of Figure S4A. (G) Yeast whole cell extracts from histone shuffle strains carrying WT H2B and H2BK123R mutant plasmids and from the *set1*Δ strain were subjected to immunoblot analyses with indicated antibodies.

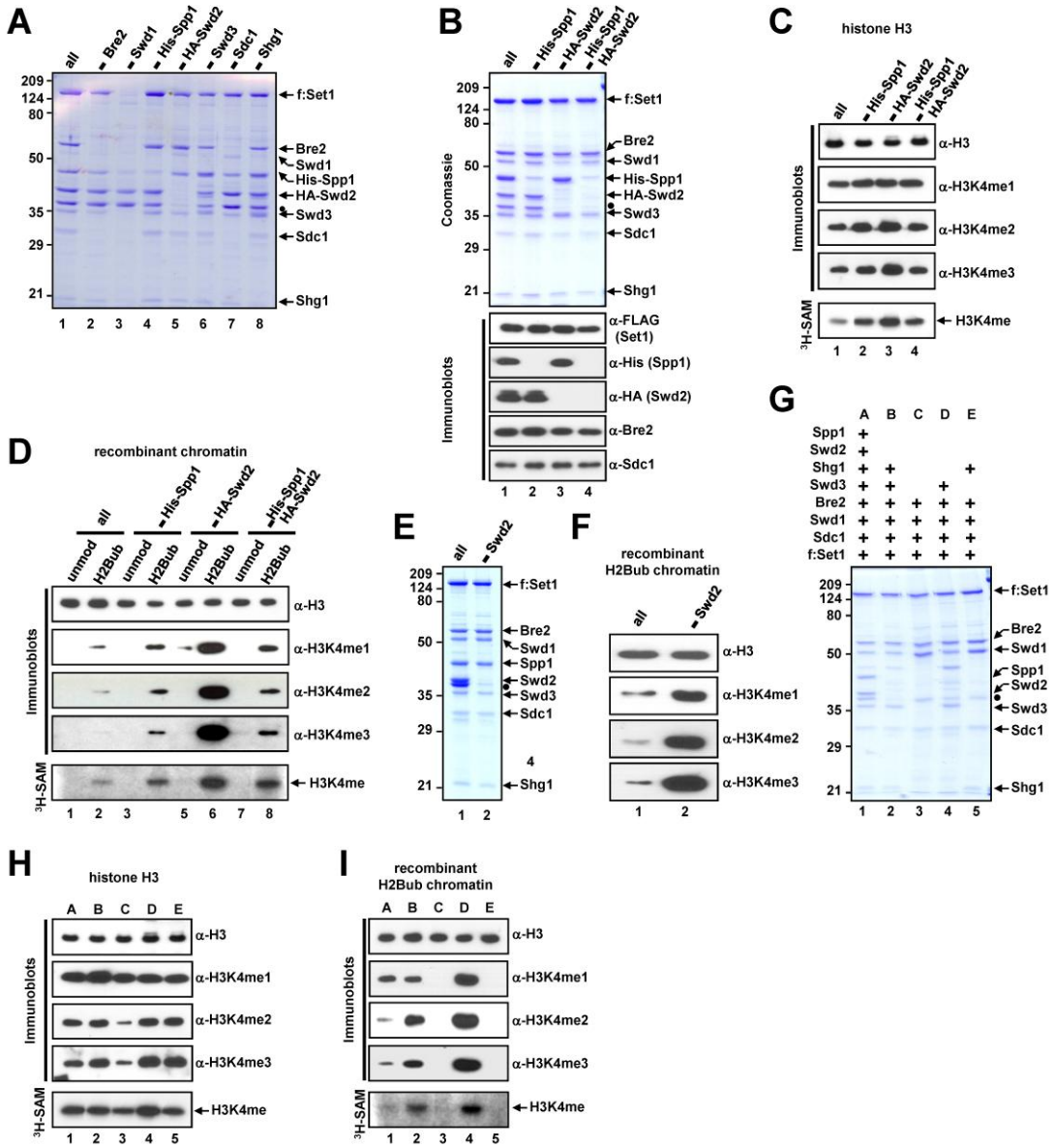
A
all
- Bre2
- Swd1
- His-Spp1
- HA-Swd2
- Swd3
- Sdc1
- Shg1
209
124
80
50
35
29
21
f:Set1
Bre2
Swd1
His-Spp1
HA-Swd2
Swd3
Sdc1
Shg1
1 2 3 4 5 6 7 8
B
all
- His-Spp1
- HA-Swd2
- His-Spp1 HA-Swd2
Coomassie
Immunoblots
α-FLAG (Set1)
α-His (Spp1)
α-HA (Swd2)
α-Bre2
α-Sdc1
1 2 3 4
C
histone H3
Immunoblots
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
3H-SAM
H3K4me
1 2 3 4
D
recombinant chromatin
unmod
H2Bub
Immunoblots
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
3H-SAM
H3K4me
1 2 3 5 6 7 8
E
all
- Swd2
f:Set1
Bre2
Swd1
Spp1
Swd2
Swd3
Sdc1
Shg1
1 2
F
recombinant H2Bub chromatin
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
1 2
G
A B C D E
Spp1
Swd2
Shg1
Swd3
Bre2
Swd1
Sdc1
f:Set1
1 2 3 4 5
H
histone H3
A B C D E
Immunoblots
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
3H-SAM
H3K4me
1 2 3 4 5
I
recombinant H2Bub chromatin
A B C D E
Immunoblots
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
3H-SAM
H3K4me
1 2 3 4 5

Figure S2

Figure S2. Minimal Subunit Requirement for Yeast Set1 Complex-Mediated H3K4 Methylation, Related to Figure 2

(A) Lowered yields of purified complexes in the absence of specific subunits. Equal volumes of purified complexes from Figure 2A were analyzed by SDS-PAGE/Coomassie Blue staining. Note that the Set1 level is decreased in the absence of Swd1, Swd3, Bre2, Sdc1 or Swd2.

(B-D) Dispensability of Swd2 and Spp1 for ySet1C (containing full-length Set1)-mediated H2Bub-dependent H3K4 methylation in vitro. (B) SDS-PAGE/Coomassie Blue staining and immunoblots of purified ySet1Cs reconstituted with baculoviruses expressing FLAG-Set1, Swd1, Swd3, Bre2, Sdc1, Shg1 in the presence and in the absence of His-Spp1 and HA-Swd2 as indicated. (C and D) In vitro HMT assays with indicated ySet1Cs from (B) and either free histone H3 (C), chromatin arrays assembled with unmodified or H2Bub octamers (D) as substrates.

(E and F) Enhanced H3K4 methylation activity of ySet1C lacking Swd2 (Figure 2C) is independent of the HA tag on Swd2. (E) SDS-PAGE/Coomassie Blue staining of the purified ySet1Cs containing all (untagged) seven ySet1C subunits and lacking Swd2. (F) In vitro HMT assay with purified ySet1Cs from (E) and chromatin substrate assembled with the H2Bub octamer.

(G-I) Identification of Swd1, Swd3, Bre2 and Sdc1 as the minimal set of subunits required for H2Bub-dependent H3K4 methylation by ySet1C (containing full-length Set1). (G) SDS-PAGE/Coomassie Blue staining of the purified ySet1Cs reconstituted with indicated combinations of baculoviruses expressing ySet1C subunits. Labels for each complex used for assays in (H) and (I) are indicated at the top. Free histone H3 (H) and chromatin arrays assembled with H2Bub octamer (I) were subjected to in vitro HMT assays with purified ySet1Cs from (G).

Note that, in (B), (E) and (G), complex loadings were normalized to FLAG-Set1 and that the dot indicates the degraded N-terminal part of the Set1 polypeptide.

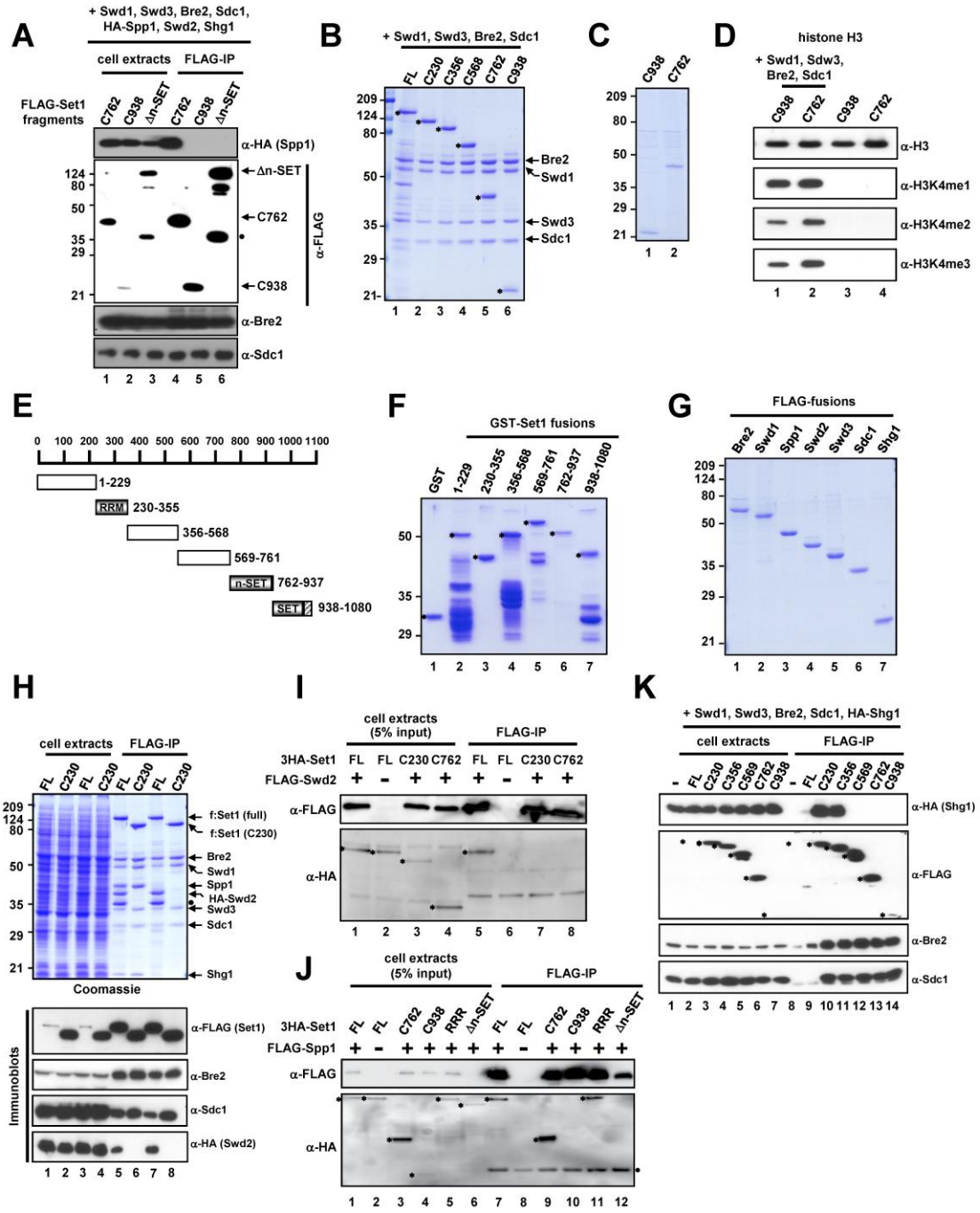
A
+ Swd1, Swd3, Bre2, Sdc1, HA-Spp1, Swd2, Shg1
cell extracts
FLAG-IP
FLAG-Set1 fragments
C762
C938
Δn-SET
α-HA (Spp1)
Δn-SET
C762
C938
α-FLAG
α-Bre2
α-Sdc1
B
+ Swd1, Swd3, Bre2, Sdc1
FL
C230
C356
C568
C762
C938
Bre2
Swd1
Swd3
Sdc1
C
C938
C762
D
histone H3
+ Swd1, Sdw3, Bre2, Sdc1
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
E
1-229
RRM
230-355
356-568
569-761
n-SET
762-937
SET
938-1080
F
GST-Set1 fusions
GST
1-229
230-355
356-568
569-761
762-937
938-1080
G
FLAG-fusions
Bre2
Swd1
Spp1
Swd2
Swd3
Sdc1
Shg1
H
cell extracts
FLAG-IP
f:Set1 (full)
f:Set1 (C230)
Bre2
Swd1
Spp1
HA-Swd2
Swd3
Sdc1
Shg1
Coomassie
Immunoblots
α-FLAG (Set1)
α-Bre2
α-Sdc1
α-HA (Swd2)
I
cell extracts (5% input)
FLAG-IP
3HA-Set1
FLAG-Swd2
α-FLAG
α-HA
J
cell extracts (5% input)
FLAG-IP
3HA-Set1
FLAG-Spp1
RRR
α-FLAG
α-HA
K
+ Swd1, Swd3, Bre2, Sdc1, HA-Shg1
cell extracts
FLAG-IP
α-HA (Shg1)
α-FLAG
α-Bre2
α-Sdc1

Figure S3

Figure S3. Subunit Interactions with Set1, Related to Figure 3

(A) Spp1 interacts with the n-SET domain. Insect cells were co-infected with combinations of baculoviruses expressing indicated Set1 fragments and ySet1C subunits (Swd1, Swd3, Bre2, Sdc1, Swd2, Shg1 and HA-Spp1). Total cell extracts and complexes purified on M2 agarose (FLAG-IP) were analyzed by immunoblot with indicated antibodies. Note that the fragments lacking the n-SET domain (C938 and ∆n-SET) fail to co-immunoprecipitate HA-Spp1.

(B) The SET and post-SET domains are sufficient to stably interact with Swd1, Swd3, Bre2 and Sdc1. SDS-PAGE/Coomassie Blue staining of purified ySet1Cs reconstituted with baculoviruses expressing FLAG-tagged Set1 or Set1 fragments and Swd1, Swd3, Bre2 and Sdc1. FLAG-Set1 polypeptides are marked by asterisks. Complex loading was normalized to Swd1, Swd3, Bre2 and Sdc1. Note: FL, full-length Set1 (in this and other figures).

(C and D) The SET and post-SET domains associated with Swd1, Swd3, Bre2 and Sdc1 are sufficient to methylate free histone H3. (C) SDS-PAGE/Coomassie Blue staining of purified FLAG-C938 and C762 (single) polypeptides. (D) In vitro HMT assays with free histone H3 and either ySet1Cs reconstituted with baculoviruses expressing FLAG-C938 or FLAG-C762 Set1 and Swd1, Swd3, Bre2 and Sdc1 from (B) (lanes 1 and 2) or with baculovirus-expressed FLAG-C938 or FLAG-C762 Set1 single polypeptides from (C) (lanes 3 and 4).

(E-G) Analyses of purified proteins by SDS-PAGE with Coomassie Blue staining. (E) A schematic diagram of Set1-derived fragments used for protein interaction studies in Figure 3D. Note that because of the absence of any identified motif, fragments encoding Set1 residues 1-229, 356-568 and 569-761 are depicted as blank boxes. (F) GST and GST-Set1 fragments expressed and purified from bacteria. Encoded amino acids within the Set1 fragments are indicated. Intact polypeptides are marked by asterisks. (G) FLAG-tagged individual ySet1C subunits expressed and purified via the baculovirus expression systems.

(H and I) Swd2 associates with N-terminal residues 1-229 of Set1. (H) Insect cells were co-infected with combinations of baculoviruses expressing FLAG-tagged full-length (FL) or C230 Set1, as indicated, and either seven ySet1C subunits (Swd1, Swd3, Bre2, Sdc1, Spp1, Shg1 and HA-Swd2; lanes 1, 2, 5 and 6) or five ySet1C subunits (Swd1, Swd3, Bre2, Sdc1 and HA-Swd2; lanes 3, 4, 7 and 8). Total cell extracts and complexes purified on M2 agarose (FLAG-IP) were analyzed by SDS-PAGE/Coomassie Blue staining and by immunoblots with indicated antibodies. Due to a low expression level of FL Set1 (anti-FLAG immunoblot, lanes 1 and 3 versus lanes 2 and 4) that results in low complex recovery, loadings for purified complexes were normalized to Swd1, Swd3, Bre2 and Sdc1 (lanes 5-8). (I) Plasmids carrying FLAG-Swd2 were introduced into yeast strains that contain chromosomal genes expressing triple HA-tagged FL, C230 or C762 Set1 proteins**.** Yeast whole cell extracts and complexes purified on M2 agarose (FLAG-IP) were analyzed by immunoblots with indicated antibodies. HA-Set1 polypeptides are marked by asterisks. Consistent with the in vitro binding results (Figure 3D), an intracellular interaction of N-terminal FLAG-tagged Swd2 with HA-tagged FL Set1, but not HA-tagged C230 (lacking the Set1 N-terminus), was detected by coimmunoprecipitation with anti-FLAG antibody (lane 5 versus lane 7).

(J) Spp1 associates with the n-SET domain (residues 762-937) of Set1. Plasmids carrying FLAG-Spp1 were introduced into yeast strains that contain chromosomal genes expressing triple HA-tagged FL, C230, C762, RRR or ∆n-SET Set1 proteins (Figures 4B and 5D). An intracellular interaction of N-terminal FLAG-tagged Spp1 with HA-tagged Set1 fragments were analyzed as in (I).

(K) Shg1 associates with residues 356-568 of Set1. Insect cells were co-infected with combinations of baculoviruses expressing FLAG-tagged Set1 or Set1 fragments and ySet1C subunits (Swd1, Swd3, Bre2, Sdc1 and HA-Shg1). Total cell extracts and complexes purified on M2 agarose (FLAG-IP) were analyzed by immunoblots with indicated antibodies. FLAG-Set1 polypeptides are marked by asterisks. Note that inefficient subunit coimmunoprecipitation in lane 9 is due to low expression level of FL Set1 (anti-FLAG immunoblot, lane 2 versus lanes 3-7; see also Figure S3H).

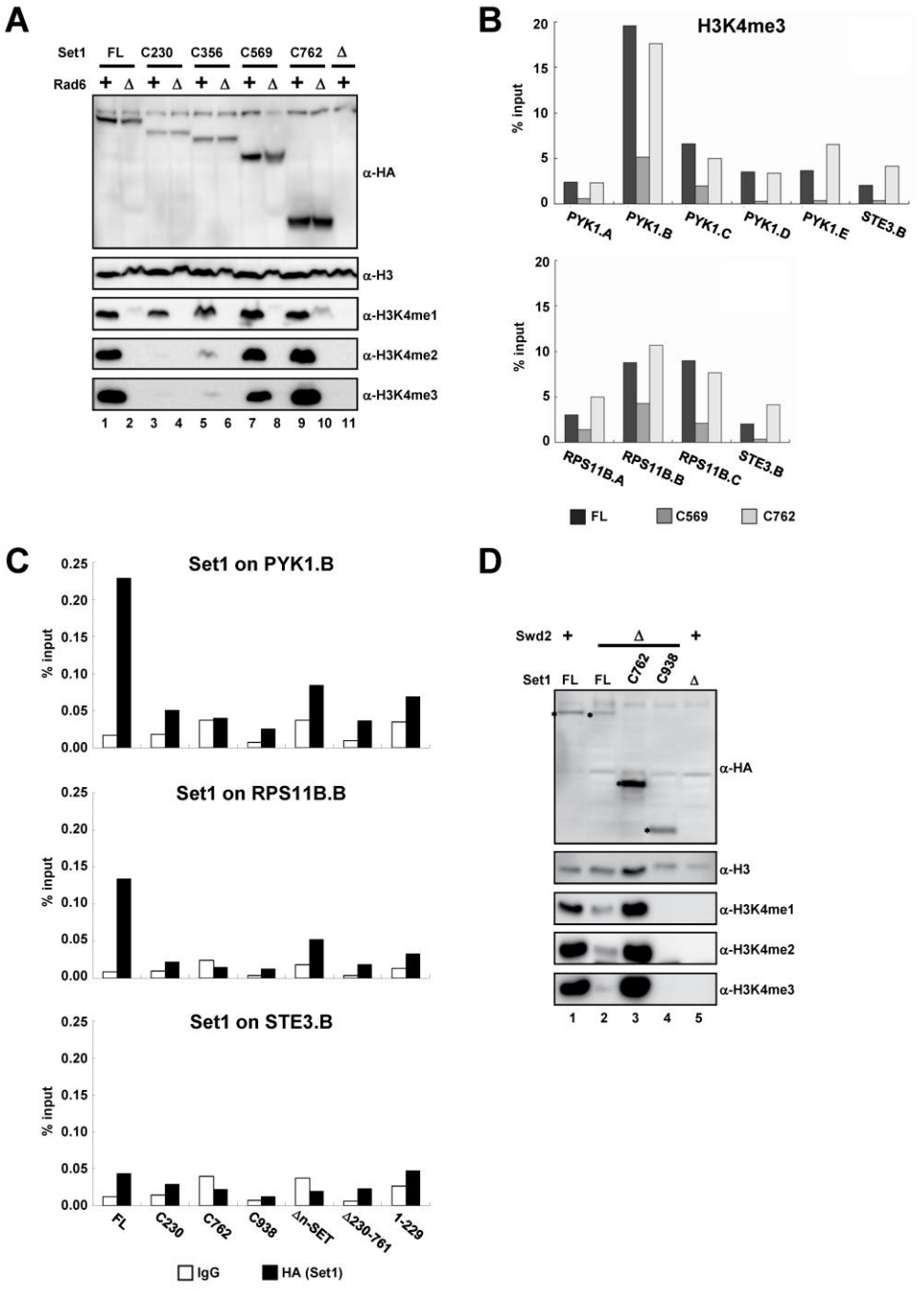
A
Set1 FL C230 C356 C569 C762 Δ
Rad6 + Δ + Δ + Δ + Δ + Δ +
α-HA
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
1 2 3 4 5 6 7 8 9 10 11
B
H3K4me3
% input
PYK1.A PYK1.B PYK1.C PYK1.D PYK1.E STE3.B
RPS11B.A RPS11B.B RPS11B.C STE3.B
FL C569 C762
C
Set1 on PYK1.B
Set1 on RPS11B.B
Set1 on STE3.B
% input
FL C230 C762 C938 Δn-SET Δ230-761 1-229
IgG HA (Set1)
D
Swd2 + Δ +
Set1 FL FL C762 C938 Δ
α-HA
α-H3
α-H3K4me1
α-H3K4me2
α-H3K4me3
1 2 3 4 5

Figure S4

Figure S4. Immunoblots and ChIP Analyses with Yeast Strains Containing Set1 Derivatives, Related to Figure 4

(A) Yeast whole cell extracts from different *set1* mutants that carry indicated chromosomal genes expressing HA-tagged Set1 or Set1 fragments and their *rad6*Δ derivatives were subjected to immunoblot analyses with indicated antibodies.

(B and C) ChIP analyses were performed on the *PYK1*, *RPS11B* and *STE3* loci schematized in Figure 4D. (B) H3K4me3 ChIP analyses on the indicated genes/amplicons in yeast strains carrying chromosomal HA-tagged FL, C569 or C762 Set1 genes. (C) ChIP analyses with anti-HA antibody to determine chromatin association of HA-tagged Set1 or Set1 fragments at 5'-transcribed regions (marked 'B' in Figure 4D). Anti-rabbit IgG antibody was used as a control. Note: Δ230-761, HA-Set1 fragment with residues 230-761 deletion; 1-229, HA-Set1 fragment encoding residues 1-229.

(D) Levels of HA-Set1 and H3K4me1/2/3 in the *swd2* deletion strains. Yeast whole cell extracts from different *set1* mutants that carry the indicated chromosomal genes expressing HA-tagged Set1 or Set1 fragments in the *swd2* deletion background (Nedea et al., 2008) were subjected to immunoblot analyses with indicated antibodies. HA-tagged Set1 polypeptides are marked by asterisks.

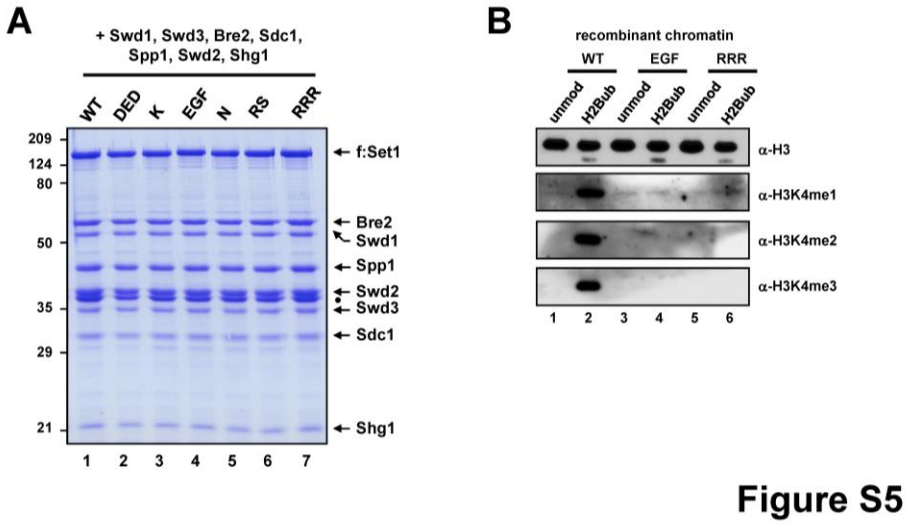

Figure S5

Figure S5. Reconstitution and Purification of the Yeast Set1 Complexes Harboring Mutations within the n-SET Domain, Related to Figure 5

(A) SDS-PAGE/Coomassie Blue staining of purified ySet1Cs reconstituted with baculoviruses expressing indicated FLAG-tagged WT and Set1 mutants and all seven of the other (untagged) subunits. Protein loading was normalized to FLAG-Set1.

(B) Chromatin templates assembled with unmodified or H2Bub octamer were subjected to in vitro HMT assays with indicated purified ySet1Cs.

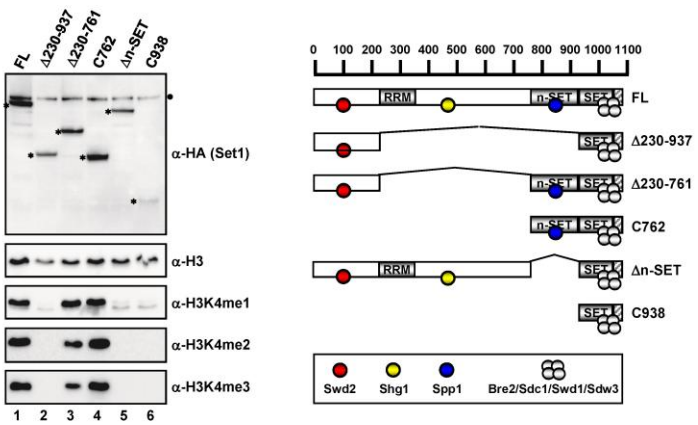

Figure S6

Figure S6. N-terminal Residues (1-229) within Set1 and Its Associated Subunit Swd2 Do Not Mediate H2B Ubiquitylation-Dependent H3K4 Methylation, Related to Figure 6

(Left) Whole cell extracts from yeast cells that carry chromosomal genes expressing the indicated HA-tagged FL Set1 or Set1 fragments were subjected to immunoblot analyses with the indicated antibodies. HA-Set1 polypeptides are marked by asterisks. The dot indicates a non-specific band. Note that Δ230-937 Set1 [consisting of N-terminal residues (1-229), and the SET and the post-SET domains (938-1080)] is unable to rescue H3K4 methylation (lane 2). This indicates that Set1 residues (1-229) and the associated Swd2 cannot replace the role of the n-SET domain and Spp1 in mediating H2Bub-dependent H3K4 methylation. (Right) Schematic diagram of Set1 fragments used in the left panel with their associated subunits based on the structural organization model in Figure 3E. Note: Δ230-937, HA-Set1 fragment with residues 230-937 deletion; Δ230-761, HA-Set1 fragment with residues 230-761 deletion.

SUPPLEMENTAL TABLES

Table S1. Yeast Strains Used in This Study, Related to Figures 4, 5 and 6

Strain name	Genotype	Reference
YJK427	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-*SET1*::HphMX	This study
YJK428	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C230-*SET1*::HphMX	This study
YJK444	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C356-*SET1*::HphMX	This study
YJK445	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C569-*SET1*::HphMX	This study
YJK430	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX	This study
YJK431	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C938-*SET1*::HphMX	This study
YJK496	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-ΔnSET-*SET1*::HphMX	This study
YJK523	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-mutE(R881A, S884A)-*SET1*::HphMX	This study
YJK524	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-mutF(R904A, R908A, R909A) -*SET1*::HphMX	This study
YJK525	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-mutG(L927A, L928A)-*SET1*::HphMX	This study
YJK526	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-mutH(N931A, Q932A, L933A)-*SET1*::HphMX	This study
YJK487	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-*SET1*::HphMX, *rad6*Δ::KanMX	This study
YJK488	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C230-*SET1*::HphMX, *rad6*Δ::KanMX	This study
YJK489	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C356-*SET1*::HphMX, *rad6*Δ::KanMX	This study
YJK490	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C569-*SET1*::HphMX, *rad6*Δ::KanMX	This study
YJK491	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *rad6*Δ::KanMX	This study
YJK529	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-Δ(231-937)-*SET1*::HphMX	This study
YJK528	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0,3HA-Δ(231-761)-*SET1*::HphMX	This study
YJK539	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::KanMX	Open Biosystems
YJK556	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-*SET1*::HphMX, p[2μ, *URA3*, GAL1p::FLAG-*SWD2*]	This study
YJK557	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-*SET1*::HphMX, p[2μ, *URA3*]	This study
YJK558	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C230-*SET1*::HphMX, p[2μ, *URA3*, GAL1p::FLAG-*SWD2*]	This study
YJK559	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, p[2μ, *URA3*, GAL1p::FLAG-*SWD2*]	This study
YJK560	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-SET1::HphMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1*]	This study
YJK561	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1*]	This study
YJK562	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C938-*SET1*::HphMX, p[2μ, URA3, GAL1p::FLAG-*SPP1*]	This study

YJK346	*MAT*a, *hht1-hhf1Δ*::KanMX, *hhf2-hht2Δ*::NatMX, *hta1-htbΔ*::HphMX, *hta2-htb2Δ*::NatMX, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, p[CEN *LEU2 HTA1-HTB1 HHT2-HHF2*]	(Ahn et al., 2005), This study
YJK353	*MAT*a, *hht1-hhf1Δ*::KanMX, *hhf2-hht2Δ*::NatMX, *hta1-htbΔ*::HphMX, *hta2-htb2Δ*::NatMX, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, p[CEN *LEU2 HTA1-htb1*K123R *HHT2-HHF2*]	(Ahn et al., 2005), This study
YJK600	*swd2*Δ::KanMX, pGPF-*sen1*[BamHI-Sau3 fragment]-*URA3*, 3HA-*SET1*::HphMX	(Nedea et al., 2008), This study
YJK601	*swd2*Δ::KanMX, pGPF-*sen1*[BamHI-Sau3 fragment]-*URA3*, 3HA-C762-*SET1*::HphMX	(Nedea et al., 2008), This study
YJK602	*swd2*Δ::KanMX, pGPF-*sen1*[BamHI-Sau3 fragment]-*URA3*, 3HA-C938-*SET1*::HphMX	(Nedea et al., 2008), This study

Table S2. Oligonucleotide Sequences for ChIP Analysis, Related to Figures 4 and 5

Target Region (bp from the start of the gene)	Primer Name	Sequence
PYK1.A (-297/-153)	PYK1 p3 PYK1 p4	CCTTTCCTTCCCATATGATGCTA AAGGGGACCATGATATAACTGGA
PYK1.B (+195/+294)	PYK1 p1 PYK1 p2	CAACGCCAGAAAGTCCGAAGAA TTGGTGGGATTGGGTAGTCAACA
PYK1.C (+750/+916)	PYK1 p5 PYK1 p6	CGACGAAATCTTGAAGGTCACTG GCTCTGGTTGGTCTTGGGTTGTA
PYK1.D (+1106/+1274)	PYK1 p7 PYK1 p8	GAAACTGTACTCCAAAGCCAACCT CTGGTAACCAAGATGATTGGACA
PYK1.E (+2008/+2174)	PYK1 p9 PYK1 p10	TCGCGTTTCAAGATTTCAAAGG GCCACTTCAGTTTTCTTCCCAT
RPS11B.A (-276/-14)	RPS11B p3 RPS11B p4	TTTCCCGCTTTGTTTTTATTCCAC GATACGTTTCCTCTAAGTCTTCAGC
RPS11B.B (+219/+425)	RPS11B p1 RPS11B p2	ATGATTGAGATTTCGTTACACAGT AGATCCTCCTTACTTGGCATTAG

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

cDNA, Plasmids, Baculoviruses, Recombinant Proteins and Complex

The cDNAs for yeast Set1 (Gene ID: 529116), Bre2 (1360314), Swd1 (6319320), Spp1 (6325119), Swd2 (6322834), Swd3 (6319651), Sdc1 (6320677) and Shg1 (6319735) were PCR-amplified from yeast genomic DNA (Gibco-Invitrogen). For expression of FLAG-tagged proteins in yeast, cDNAs were subcloned into pGALL-pRS426 (ATCC). For GST-tagged and His-tagged proteins, cDNAs were subcloned into pGEX4T (Amersham) and pET28 (Novagen), respectively, expressed in *E. coli*, and purified on glutathione-Sepharose 4B beads (Amersham) and Ni-NTA beads (Qiagen), respectively. For baculovirus-mediated expression, cDNAs were subcloned in pFASTBAC1 with or without an epitope tag and baculoviruses were generated according to the manufacturer's instruction (Gibco-Invitrogen). For expression of FLAG-tagged Set1C subunits, and for reconstitution of Set1 complexes containing FLAG-Set1 or FLAG-Set1 fragments, Sf9 cells were infected with combinations of baculoviruses and proteins/complexes were affinity purified on M2 agarose (Sigma) as described (Kim et al., 2010). The expression and purification of *Xenopus laevis* recombinant histones, semisynthetic H2Bub and histone octamers were as described (McGinty et al., 2008; McGinty et al., 2009).

In Vitro Chromatin Assembly for Methyltransferase Assays

Procedures for chromatin assembly with the recombinant ACF system were adapted from Ito et al. (1999). Briefly, the reaction containing core histone octamer (700 ng) and NAP1 (5 μg) in 55 μl HEG buffer (25 mM HEPES [pH 7.6], 0.1 mM EDTA and 10 % glycerol) was incubated on ice for 30 min. After the further addition of Acf1 (60 ng), ISW1 (80 ng) and plasmid (700 ng, p53ML [Kim et al., 2010]), the reaction was adjusted to 25 mM HEPES [pH 7.6], 0.1 mM EDTA, 10 % glycerol, 50 mM KCl, 3.4 mM ATP and 4.8 mM $MgCl_2$ in a final volume of 70 μl and incubated at 27 °C for 3 h.

In Vitro Histone Methyltransferase Assays

For free histone and oligonucleosome methyltransferase assays, reactions containing 100 ng recombinant histone H3 or 1.0 μg HeLa cell-derived oligonucleosomes and purified Set1C (containing 25 ng Bre2 subunit) in 20 μl reaction buffer (50 mM Tris-Cl [pH 8.5], 50mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 % Glycerol) supplemented with 100 μM cold (for immunoblotting) or 1 μCi ^{3}H-labelled (for fluorography) S-adenosylmethionine (SAM) were incubated at 30 °C for 1 h. For recombinant chromatin methyltransferase assays, reactions containing 350 ng (histone amount) recombinant chromatin (35 μl, assembled as described above), purified Set1C [containing 50 ng Bre2 subunit or 100 ng only for the assay in Figure 6D)

and 100 µM SAM were adjusted to 40 µl with HEG buffer and incubated at 30 °C for 2 h. Proteins were resolved by SDS-PAGE and subjected to immunoblotting (cold SAM) or autoradiography (^{3}H-labelled SAM).

Protein Interaction Assays

For GST-pull down assays, 2 µg of GST or GST-fused proteins immobilized on Glutathione-Sepharose 4B beads were incubated with 100 ng of purified factors in binding buffer (20 mM Tris-Cl [pH 7.9], 300 mM KCl, 0.2 mM EDTA, 20 % glycerol, 0.1 % NP-40, 0.2 mg/ml BSA and 0.5 mM PMSF) at 4 °C for 3 h and then beads were extensively washed with binding buffer without BSA. Bound proteins were analyzed by immunoblot. For the interaction studies in Figures S3H and S3K, after 3 days of Sf9 cell infection with baculoviruses, total cell extracts were prepared by incubating the cells in lysis buffer (20 mM Tris-Cl [pH 7.9], 300 mM KCl, 0.2 mM EDTA, 20 % glycerol, 0.1 % NP-40 and 0.5 mM PMSF). Following clarification by centrifugation, extracts were incubated with M2 agarose beads at 4 °C for 3 h and, after extensive washing of beads with the lysis buffer, bound proteins were analyzed by Coomassie Blue staining and immunoblot. For coimmunoprecipitation assays, yeast whole cell extracts (WCE) were prepared in lysis buffer (50 mM Tris-Cl [pH 7.5], 350 mM NaCl, 1 mM EDTA, and 1 % Triton X-100), incubated with M2 agarose at 4 °C for 3 h and, after extensive washing of beads with the lysis buffer, bound proteins were analyzed by immunoblot.

Whole Cell Extracts and Immunoblot Analysis

Yeast whole cell extracts (WCE) were prepared from cells in exponential growth phase either in YPD or in synthetic media minus uracil by trichloroacetic acid-extraction and then subjected to immunoblot analyses.

Chromatin Immunoprecipitation

Yeast cells in exponential phase were treated with 1 % formaldehyde for 10 min at 30 °C, followed by quenching excess formaldehyde with 125 mM glycine. Cell lysates prepared by beadbeating and sonication in lysis buffer (50 mM HEPES [pH 7.5], 1 mM EDTA, 140 mM NaCl, 1 % Triton X-100, 0.1 % sodium deoxycholate) were subjected to immunoprecipitation with antibodies coupled to Protein A or G agarose. The beads were washed twice with lysis buffer and then with buffer A (50 mM HEPES [pH 7.5], 1 mM EDTA, 500 mM NaCl, 1 % Triton X-100, 0.1 % sodium deoxycholate) and with buffer B (10 mM Tris-Cl [pH 7.5], 1 mM EDTA, 250 mM LiCl, 0.5 % NP-40, 0.5 % sodium deoxycholate). Immunoprecipitated complexes were eluted with elution buffer (50 mM Tris-Cl [pH 7.5], 1 mM EDTA, 1 % SDS) and crosslinks were

reversed by incubation for at least 6 h at 65 °C. DNA was purified by QIAquick-spin columns (Qiagen) and enrichment of DNA was measured by quantitative PCR analysis.

Antibodies

Monoclonal anti-Set1, anti-Bre2 and anti-Sdc1 antibodies were obtained from the Nagy laboratory. Monoclonal anti-HA antibody was purified from the 12CA5 hybridoma cell line. The following antibodies were obtained commercially: anti-FLAG (Sigma); anti-His (Qiagen); anti-H3 (Abcam, ab1791); anti-H3K4me1 (Abcam, ab8895); anti-H3K4me2 (Abcam, ab7766); and anti-H3K4me3 (Active Motif, AM39159).

SUPPLEMENTAL REFERENCES

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