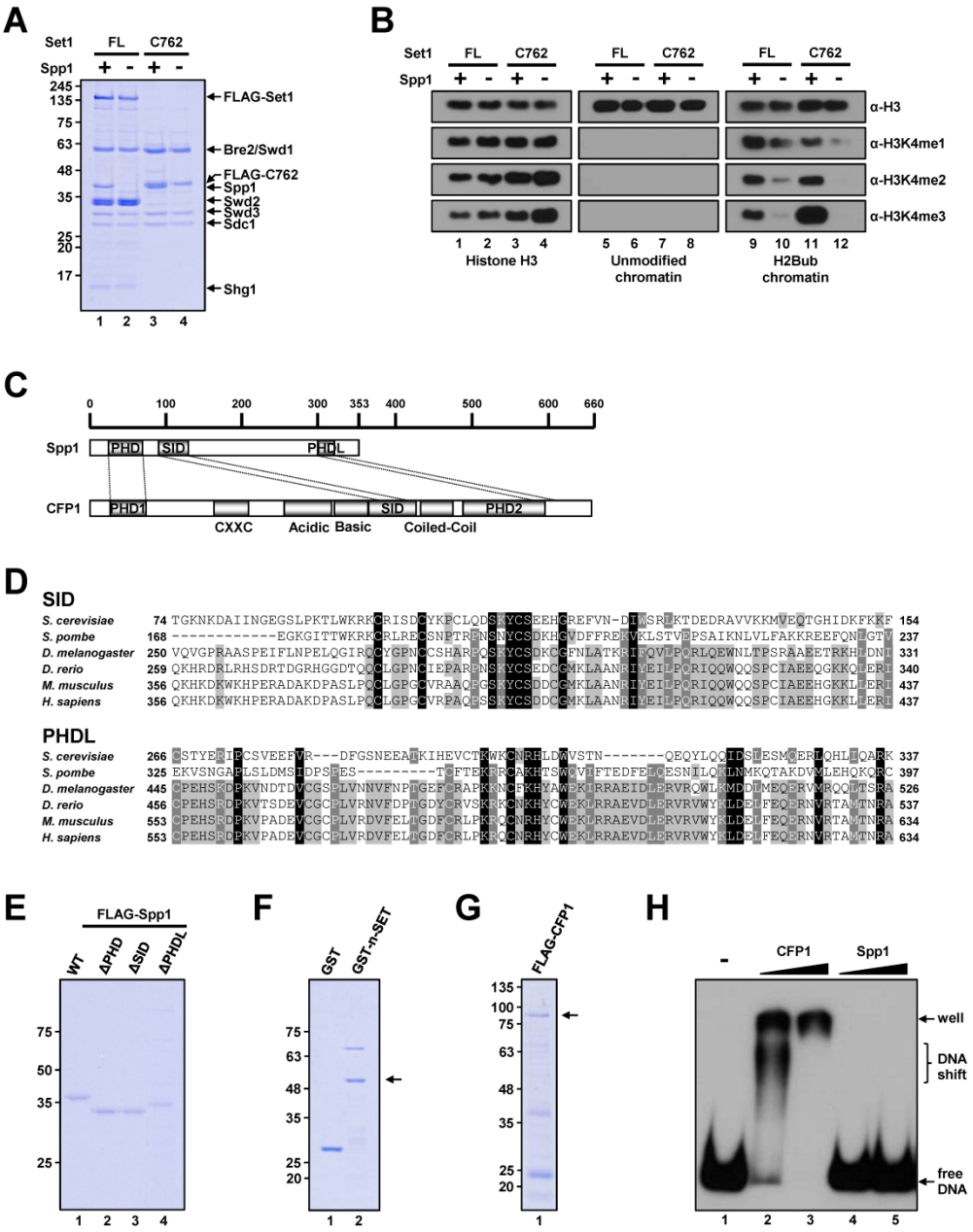
SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

Supplementary Figure S1



Supplementary Figure S1. Requirement of Spp1 for H3K4 methylation activities of the Set1 complexes and conserved domains in Spp1 family members

(**A**) SDS-PAGE/Coomassie blue staining of purified Set1Cs reconstituted with baculoviruses expressing FLAG-tagged full-length (FL) Set1 or FLAG-tagged C762 Set1 fragment and Swd1, Swd3, Bre2, Sdc1, Swd2, Shg1, and either Spp1 or a control vector.

(**B**) Requirement of Spp1 for H2Bub-dependent H3K4 methylation activities of Set1Cs containing FL Set1 or C762 Set1 fragment. Free histone H3 and recombinant chromatin templates assembled with unmodified H2B or H2Bub-containing octamers were subjected to *in vitro* histone methyltransferase (HMT) assays with purified Set1Cs as indicated. H3 methylation status was monitored by immunoblotting with indicated antibodies.

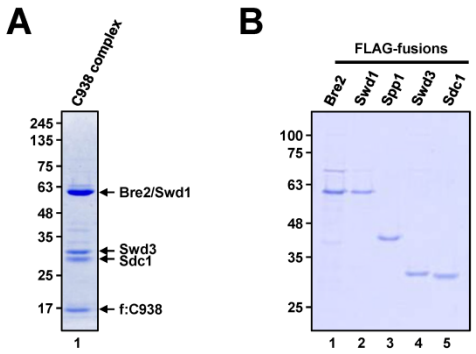
(**C**) A schematic representation of conserved domains in yeast Spp1 and human CFP1. Domain organization within CFP1 was adopted from (Butler et al., 2008). Homologous regions are depicted by dotted lines.

(**D**) Multiple sequence alignments (using ClustalW2) of the SID (top) and PHDL domain (bottom) from Spp1 family members: *Saccharomyces cerevisiae* (GenBank accession number: KZV07377.1), *Schizosaccharomyces pombe* (CAA20664.1), *Drosophila melanogaster* (Q9W352.1), *Danio rerio* (CAE30421.1), *Mus musculus* (NP_083144.1) and *Homo sapiens* (NP_001095124.1). Encoded amino acid numbers are indicated.

(**E-G**) Analyses of purified proteins by SDS-PAGE with Coomassie Blue staining. FLAG-tagged wild-type (WT) and mutant Spp1 proteins (E) and FLAG-tagged CFP1 (G) expressed and purified via the baculovirus expression systems. (F) GST and GST-tagged n-SET fragment (Set1 residues 762-937) expressed and purified from bacteria. Arrows indicate intact polypeptides.

(**H**) Comparison of DNA binding abilities of yeast Spp1 and human CFP1. Radiolabeled 601 DNA fragment (0.125 pmole) was subjected to electrophoresis mobility shift assays with purified CFP1 [0.04 pmole (lane 2) and 0.1 pmole (lane 3)] and Spp1 [0.04 pmole (lane 4) and 0.1 pmole (lane 5)] as indicated.

Supplementary Figure S2



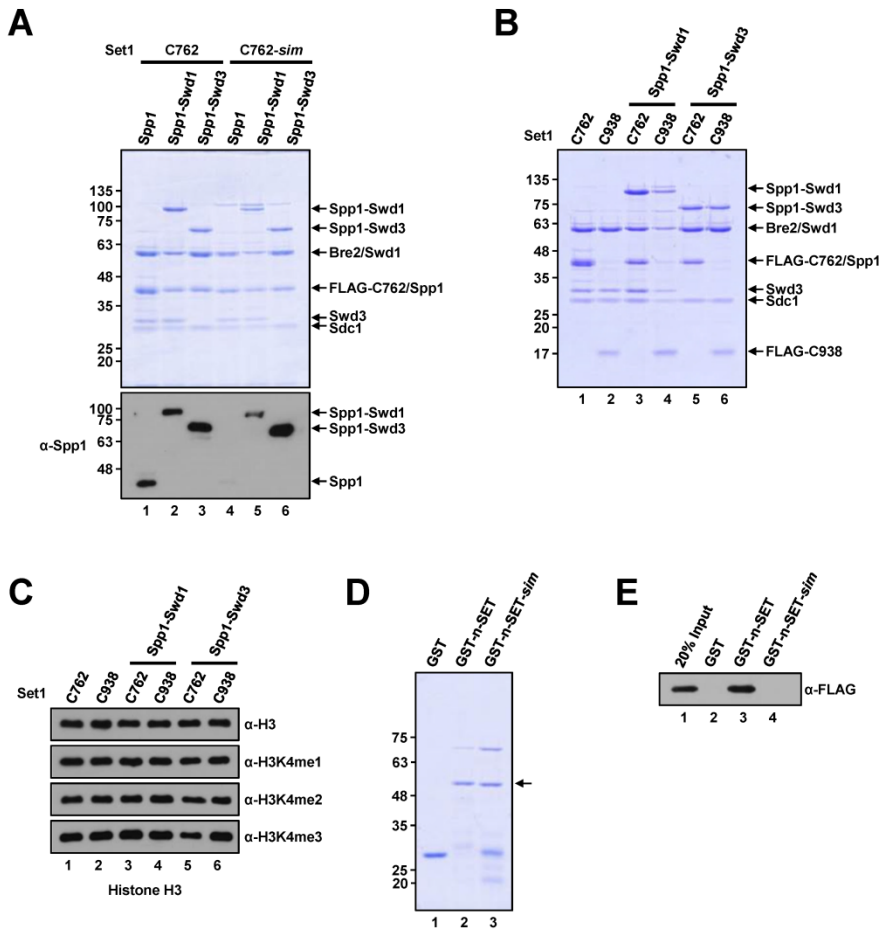
Supplementary Figure S2. Purified C938 Set1 complex and Set1 complex subunits

Analyses of purified proteins by SDS-PAGE with Coomassie Blue staining. Proteins were expressed via the baculovirus systems and purified on M2 agarose.

(**A**) The C938 Set1 complex composed of FLAG-tagged Set1 fragment (Set1 residues 938-1080 containing SET and post-SET domains) and untagged Swd1, Swd3, Bre2, and Sdc1.

(**B**) FLAG-tagged individual Set1C subunits.

Supplementary Figure S3



Supplementary Figure S3. Purified Set1 complexes with subunit fusions and mutations in the Spp1 interaction motif (SIM) and protein interaction analysis

(**A**) SDS-PAGE/Coomassie blue staining and anti-Spp1 immunoblot analyses of purified C762 Set1 and C762-*sim* Set1-based complexes containing Spp1, Spp1-Swd1 or Spp1-Swd3 fusion. Subunit compositions and subunit fusions within the complexes are depicted in Figure 3A.

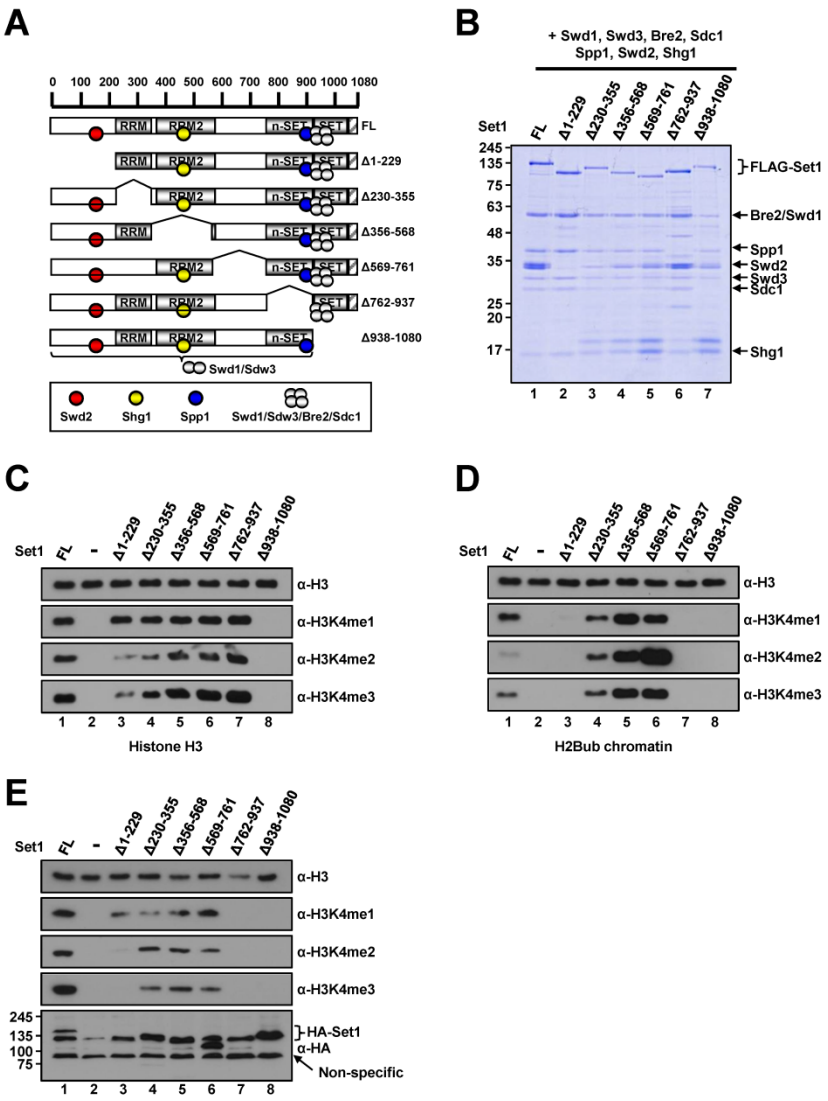
(**B**) SDS-PAGE/Coomassie blue staining analyses of purified C762 and C938-based Set1 complexes containing Spp1, Spp1-Swd1 or Spp1-Swd3 fusion.

(**C**) Free histone H3 was subjected to *in vitro* HMT assays with indicated purified C762 and C938-based Set1 complexes (Supplementary Figure S3B).

(**D**) SDS-PAGE/Coomassie blue staining of GST, GST-n-SET, and GST-n-SET-*sim* fragments expressed and purified from bacteria. Arrow indicates intact polypeptides.

(**E**) Requirement of the SIM (Spp1-interacting motif) for direct binding of Spp1 to the n-SET domain. GST pull-down assays employed purified GST-n-SET fragments (Supplementary Figure S3D) and FLAG-Spp1.

Supplementary Figure S4



Supplementary Figure S4. Purification of Spp1-containing Set1 complexes and analyses of H3K4 methylation activities

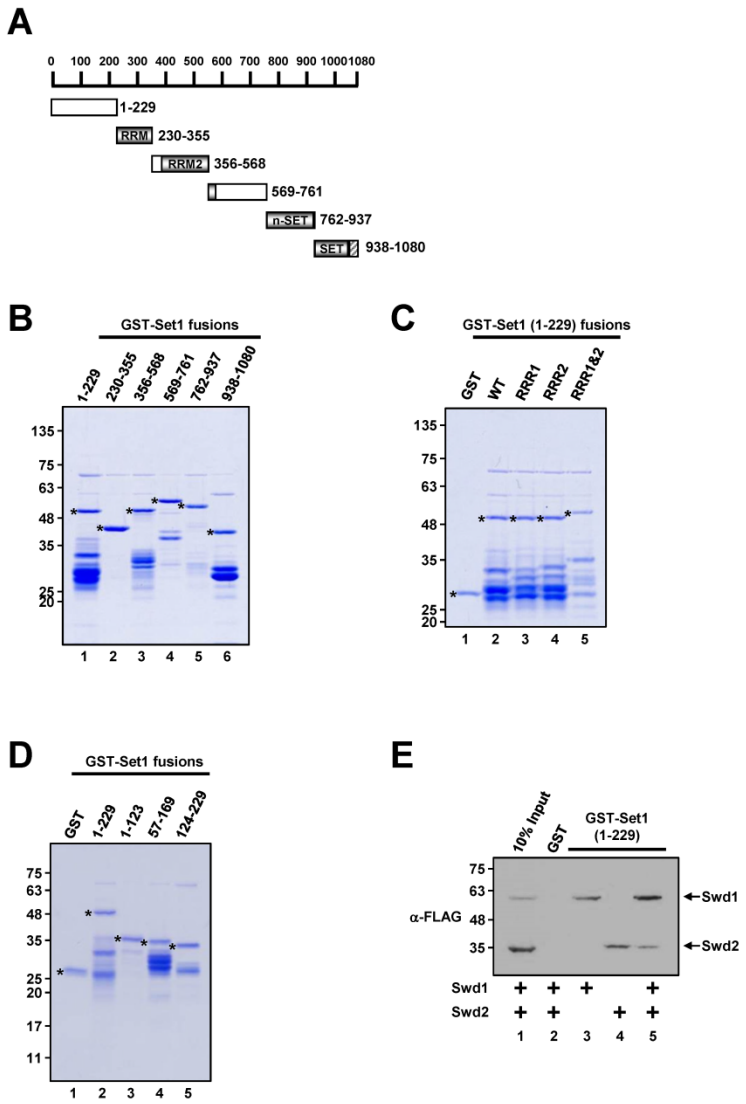
(**A**) A schematic diagram of Set1Cs containing FL Set1 and derived Set1 fragments along with associating subunits. A probable interaction between Swd1-Swd3 and a region that lies N-terminal to the SET domain (Set1 residues 1-937) in Δ938-1080 Set1 is deduced from their presence in the Δ938-1080 Set1 complex (Supplementary Figure S4B, lane 7).

(**B**) SDS-PAGE/Coomassie blue staining of purified Set1Cs reconstituted with baculoviruses expressing FLAG-Set1 or FLAG-Set1 fragments and all seven (untagged) Set1C subunits.

(**C** and **D**) Analyses of H3K4 methylation activities of purified Set1Cs. *In vitro* HMT assays with free histone H3 (C) and H2Bub chromatin (D) and indicated purified Set1Cs (Supplementary Figure S4B).

(**E**) Analyses of H3K4 methylation levels in yeast cells. Plasmids expressing indicated HA (three copies)-tagged FL Set1 or Set1 fragments were introduced into the *set1*Δ yeast strain. Yeast whole cell extracts were analyzed by immunoblots with indicated antibodies.

Supplementary Figure S5



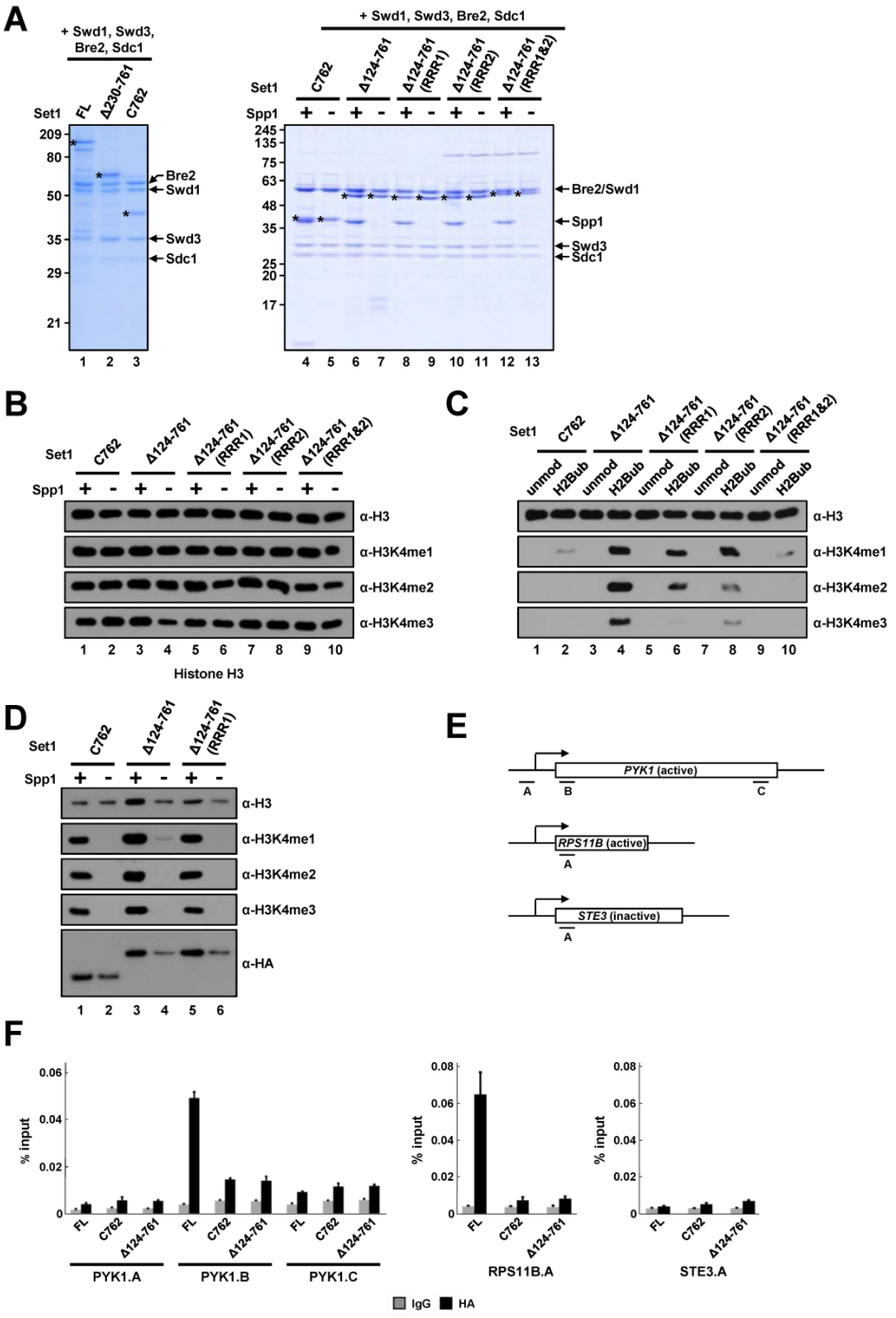
Supplementary Figure S5. Purified Set1 proteins and protein interaction analyses

(**A**) A schematic diagram of Set1-derived fragments used for protein interaction studies in Figure 5A.

(**B-D**) Analyses of purified proteins from bacteria by SDS-PAGE with Coomassie Blue staining. GST and GST-Set1 fragments used for GST-pull down assays in Figures 5A (B), 5C (C) and 5D (D). Encoded amino acids within the Set1 fragments are indicated. Intact polypeptides are marked by asterisks.

(**E**) Simultaneous direct binding of Swd1 and Swd2 to the Set1 1-229 fragment. GST pull-down assays employed GST-tagged Set1 1-229 fragment and purified FLAG-tagged Swd1 and Swd2.

Supplementary Figure S6



Supplementary Figure S6. Analyses of H3K4 methylation activities of purified Set1 complexes and ChIP analyses with yeast strains containing Set1 derivatives

(**A**) SDS-PAGE/Coomassie blue staining of purified Set1Cs reconstituted with baculoviruses expressing FLAG-Set1 or FLAG-Set1 fragments and indicated (untagged) Set1C subunits. Set1 polypeptides are marked by asterisks. Note that Swd2 and Shg1 are not included for all complex preparations.

(**B** and **C**) Analyses of H3K4 methylation activities of purified Set1Cs. Free histone H3 (B) and recombinant chromatin templates assembled with unmodified H2B or H2Bub-containing octamers (C) were subjected to *in vitro* HMT assays with purified Set1Cs (Supplementary Figure S6A) as indicated. Note that all purified complexes used in (C) do not contain Spp1. unmod, unmodified H2B.

(**D**) Analyses of H3K4 methylation levels in yeast cells. Whole-cell extracts from yeast cells that carry the indicated chromosomal genes expressing HA (three copies)-tagged Set1 fragments and their isogenic *spp1*Δ derivatives were subjected to immunoblot analyses with indicated antibodies.

(**E**) Schematic representation of transcriptionally active (*PYK1* and *RPS11B*) and inactive (*STE3*) loci and amplicons used for quantitative PCR.

(**F**) Chromatin immunoprecipitation (ChIP) analyses with anti-HA antibody on the indicated genes/amplicons (Supplementary Figure S6E) in yeast strains carrying chromosomal HA (three copies)-tagged FL, C762 or Δ124-761 Set1 genes. Anti-rabbit IgG antibody was used as a control. Error bars denote standard deviations from three technical replicates.

SUPPLEMENTARY TABLES

Supplementary Table S1. Yeast strains used in this study

Strain name	Genotype	Reference
YJK537	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX	This study
JK0202	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1*]	This study
JK0203	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1* Δ*PHD*]	This study
JK0204	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1* Δ*SID*]	This study
JK0206	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX, p[2μ, *URA3*, GAL1p::FLAG-*SPP1* Δ*PHDL*]	This study
JK0207	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX, *spp1*Δ::KanMX, p[2μ, *URA3*, GAL1p]	This study
JK0601	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX	This study
JK0523	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-C762-*SET1*]	This study
JK0529	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-*SET1*]	This study
JK0530	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(1-229)-*SET1*]	This study
JK0531	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(230-355)-*SET1*]	This study
JK0532	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(356-568)-*SET1*]	This study
JK0533	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(569-761)-*SET1*]	This study
JK0534	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(762-937)-*SET1*]	This study
JK0535	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416[SET1p::3HA-Δ(938-1080)-*SET1*]	This study
JK0536	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, *spp1*Δ::HphMX, pRS416	This study
YJK539	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX	Open Biosystems; Kim et al., 2013
JK0511	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-*SET1*]	This study
JK0512	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(1-229)-*SET1*]	This study
JK0513	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(230-355)-*SET1*]	This study
JK0514	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(356-568)-*SET1*]	This study
JK0515	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(569-761)-*SET1*]	This study
JK0516	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(762-937)-*SET1*]	This study

JK0517	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416[SET1p::3HA-Δ(938-1080)-*SET1*]	This study
JK0528	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, *set1*Δ::kanMX, pRS416	This study
YJK430	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-C762-*SET1*::HphMX	Kim et al., 2013
JK0502	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-Δ(124-761)-*SET1*::HphMX	This study
JK0503	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-Δ(124-761),RRR1-*SET1*::HphMX	This study
JK0507	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-Δ(124-761)-*SET1*::HphMX, *spp1*Δ::KanMX	This study
JK0508	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-Δ(124-761),RRR1-*SET1*::HphMX, *spp1*Δ::KanMX	This study
YJK517	*MAT*a, *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0, 3HA-*SET1*::HphMX	Kim et al., 2013

Supplementary Table S2. Oligonucleotide sequences for ChIP analysis

Target Region (bp from the start of the gene)	**Primer Name**	**Sequence**
PYK1.A (-298/-132)	PYK1.A_F	CCTTTCCTTCCCATATGATGCTA
	PYK1.A_R	AAGGGGACCATGATATAACTGGA
PYK1.B (+195/+316)	PYK1.B_F	CAACGCCAGAAAGTCCGAAGAA
	PYK1.B_R	TTGGTGGGATTGGGTAGTCAACA
PYK1.C (+1106/+1274)	PYK1.C_F	GAAACTGTACTCCAAAGCCAACCT
	PYK1.C_R	CTGGTAACCAAGATGATTGGACA
RPS11B.A (+219/+424)	RPS11B.A_F	ATGATTGAGATTTCGTTACACAGT
	RPS11B.A_R	AGATCCTCCTTACTTGGCATTAG
STE3.A (+71/+229)	STE3.A_F	GGCATTCACATACCAAGAAT
	STE3.A_R	CCTGCAACTTGATGACAATA

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

DNA electrophoretic mobility shift assays

The 601 nucleosome positioning sequence DNA was obtained from restriction of a plasmid containing tandem copies of the 601 sequence and purified by Sepharose 6 Fast Flow (GE Healthcare) gel filtration. Purified DNA was treated with the calf intestinal alkaline phosphatase (Promega) at 37 °C for 30 min to remove 5' phosphate group and then radiolabeled with [γ-32P] ATP (PerkinElmer) by the T4 polynucleotide kinase (Promega). Reactions containing radiolabeled DNA and purified Spp1 or CFP1 proteins in 40 µl reaction buffer (10 mM Tris-Cl [pH 7.5], 1 mM EDTA, 5 mM MgCl2, 50 mM K-glutamate, 5 % glycerol, and 1 mM DTT) were incubated at room temperature for 30 min. The samples were resolved by electrophoresis at 4 °C for 2 h on 5 % polyacrylamide gels in TBE buffer and subjected to autoradiography.

Chromatin immunoprecipitation

Yeast cells in exponential phase were treated with 1% formaldehyde at room temperature for 10 min and cross-linking was quenched with 125 mM glycine. Cell lysates were prepared by beadbeating and sonication in the lysis buffer (50 mM HEPES [pH 7.6], 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mg/ml bacitracin, 1 mM benzamidine, and 1 mM PMSF) and subjected to immunoprecipitation with Protein A agarose (Millipore)-coupled anti-HA antibody (Abcam) at 4°C for 1 h. The beads were washed twice with the lysis buffer and then once with the high salt buffer (50 mM HEPES [pH 7.6], 1 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mg/ml bacitracin, 1 mM benzamidine, and 1 mM PMSF) and finally once with the wash buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, and 0.5% sodium deoxycholate). Precipitated complexes were eluted by incubation with the elution buffer (50 mM Tris-Cl [pH 8.0], 1 mM EDTA, and 1% SDS) at 65°C for 15 min and then reverse-crosslinking was performed at 65°C for 6 h. Proteins were digested by incubation with 0.4 mg/ml of protease K (Sigma) at 37°C for overnight. DNA was purified by spin columns and enrichment of DNA was measured by quantitative PCR. Primers for PCR analyses are summarized in Supplementary Table S2.

SUPPLEMENTARY REFERENCES

Butler, J.S., Lee, J.H. and Skalnik, D.G. (2008) CFP1 interacts with DNMT1 independently of association with the Setd1 histone H3K4 methyltransferase complexes. *DNA Cell Biol.*, **27**, 533-543.

Kim, J., Kim, J.A., McGinty, R.K., Nguyen, U.T., Muir, T.W., Allis, C.D. and Roeder, R.G. (2013) The n-SET domain of Set1 regulates H2B ubiquitylation-dependent H3K4 methylation. *Mol. Cell*, **49**, 1121-1133.