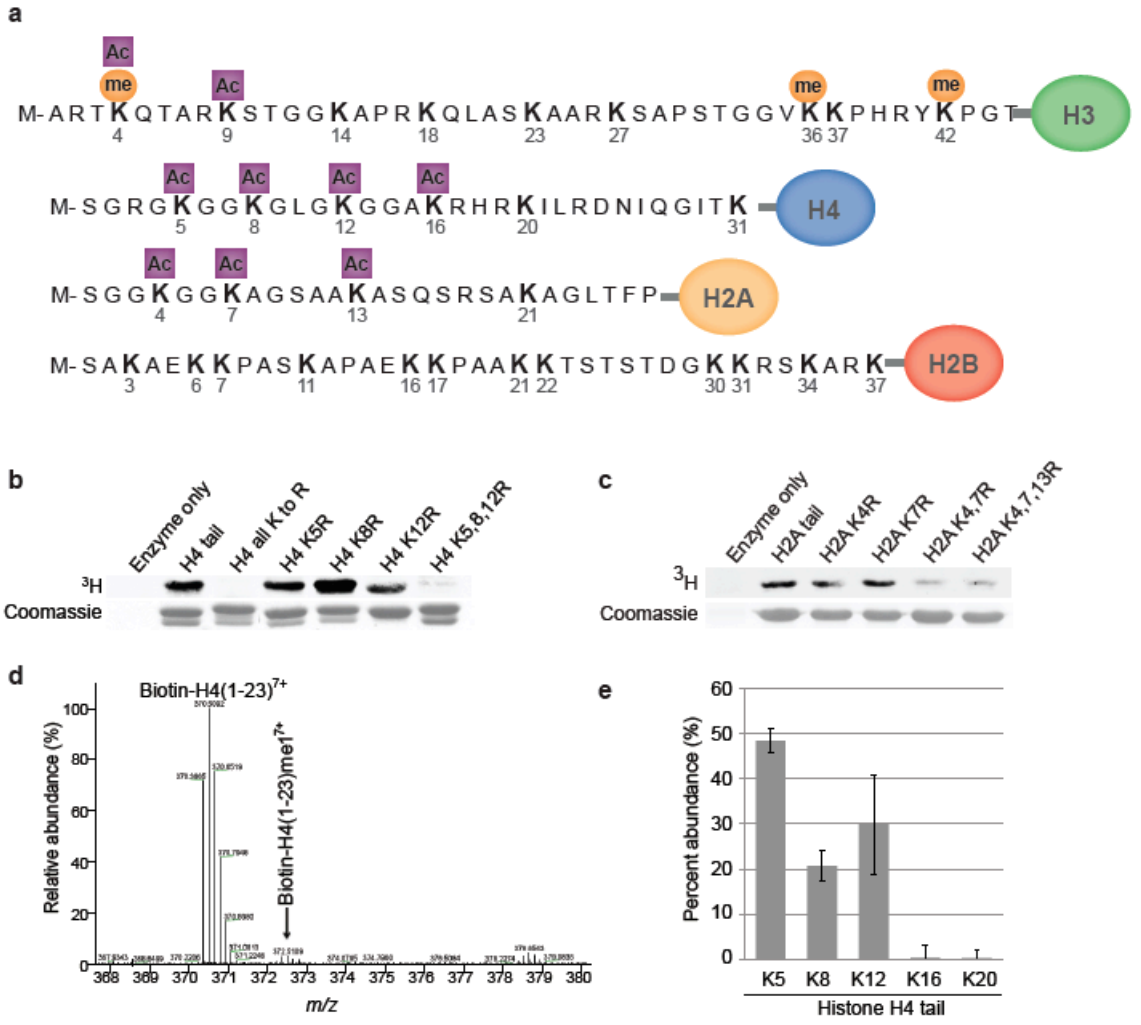
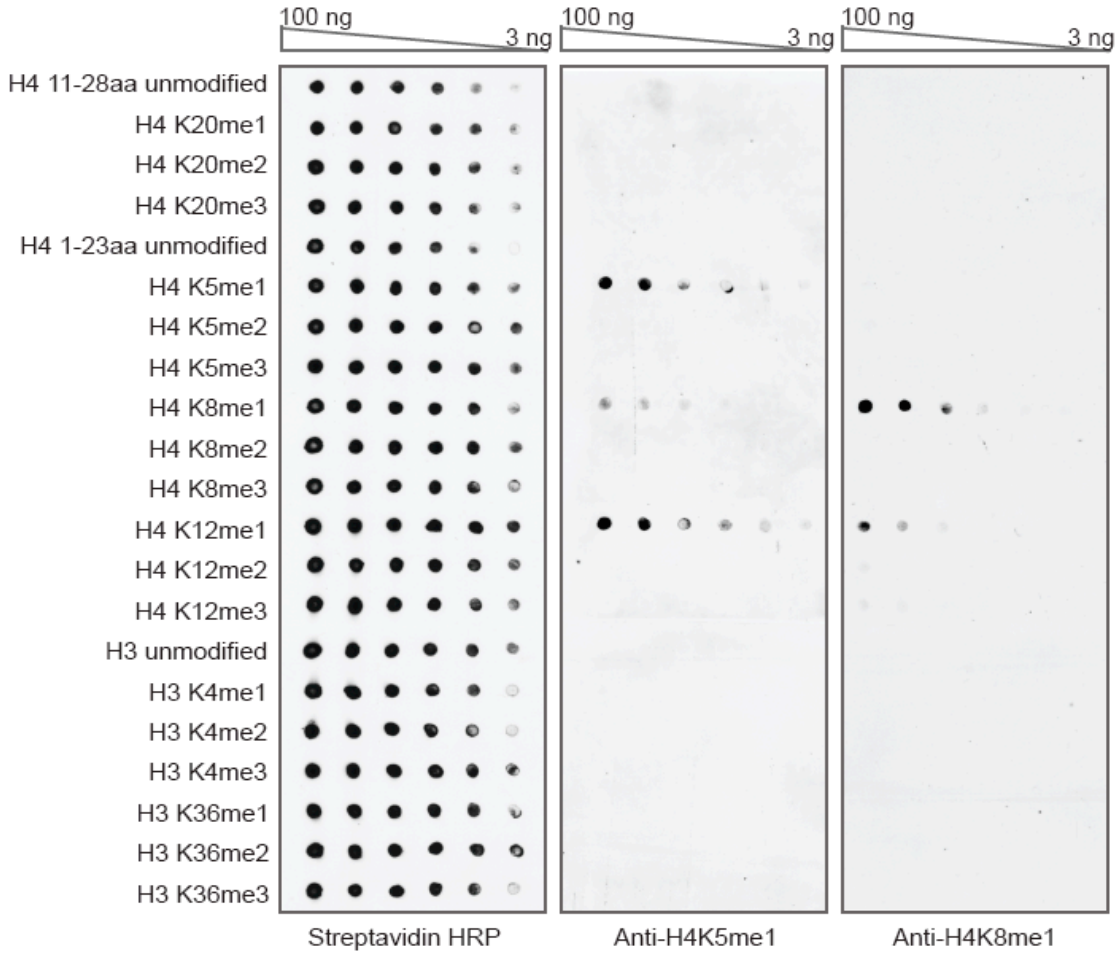
Supplementary Figures

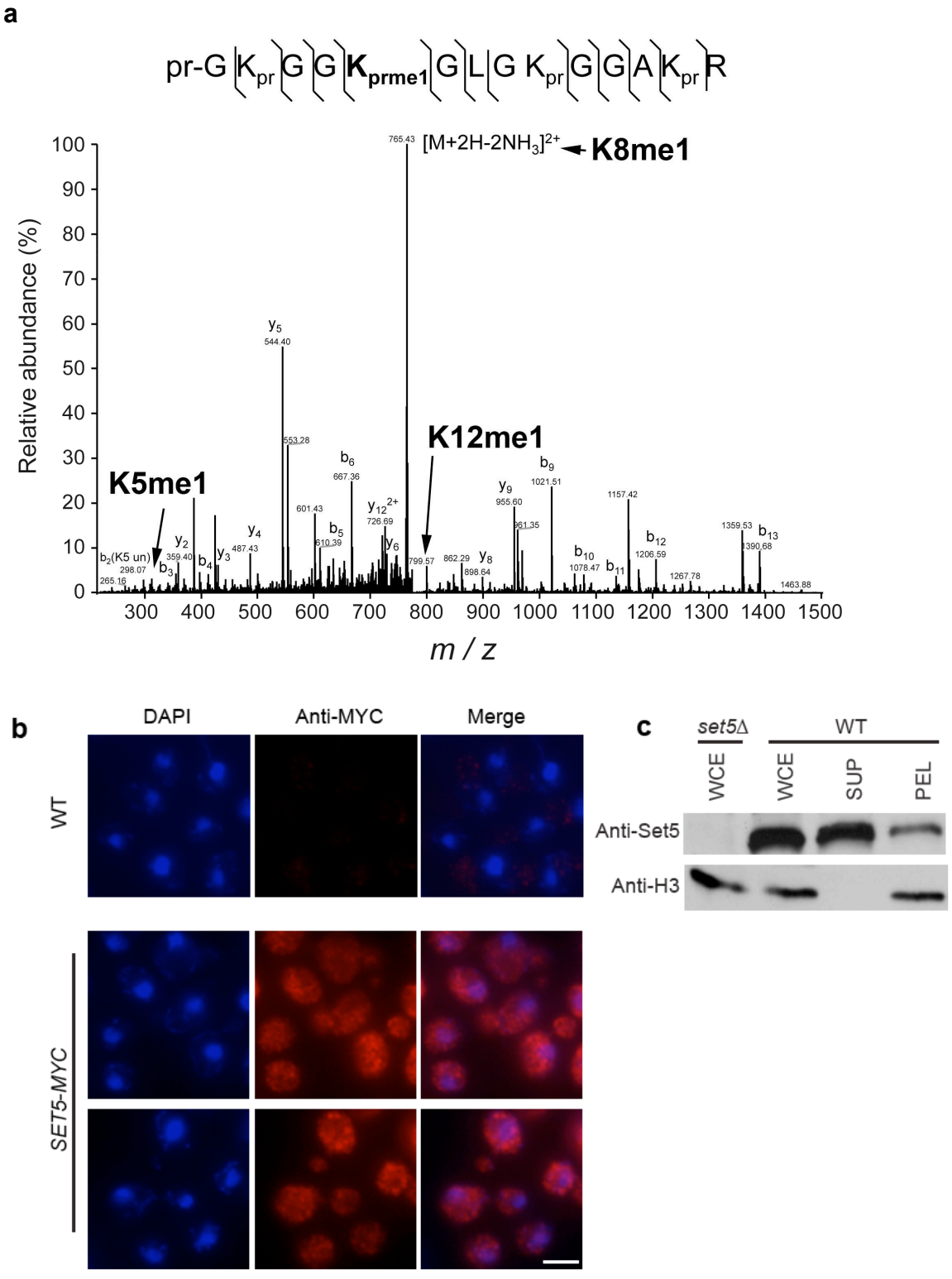


Supplementary Figure 1. Set5 methylates histones tails. (**a**) N-terminal tails of *Saccharomyces cerevisiae* histones. The sequence of the histone tails cloned for the biochemical screen are shown: H3 (amino acids 1–45), H4 (1–31), H2A (1–27), H2B (1–37). All lysines are indicated in bold and numbered. Well characterized lysine acetylation (purple) and methylation (orange) marks are also indicated. (**b**) Set5 is unable to methylate the H4K5,8,12R triple mutant. Methylation assay as in **Figure 1c** of GST-Set5 and H4 tails in which single lysines 5, 8 or 12 or the combination of all three have been mutated to arginine. (**c**) Set5 methylates lysines 4 and 7 on the histone H2A tail. Methylation assay of GST–H2A tails with the indicated point mutations. (**d**) Set5 monomethylates an H4 peptide at K5, K8 and K12. MS1 Orbitrap spectrum of the biotinylated H4 peptide (amino acids 1–23) zoomed in on the 7th charge state showing both the unmodified substrate and the monomethylated species. The peak intensities indicate an approximately 3% yield of methylated peptides. Note that the minor peaks in the region do not match the accurate mass expected for a dimethylated

species. (**e**) Histogram representing the approximate relative abundance of methylation at each site based on analysis of the MS2 spectrum (not shown). Error bars are one standard deviation based on relative peak heights of multiple fragment ions containing redundant methylation site information.

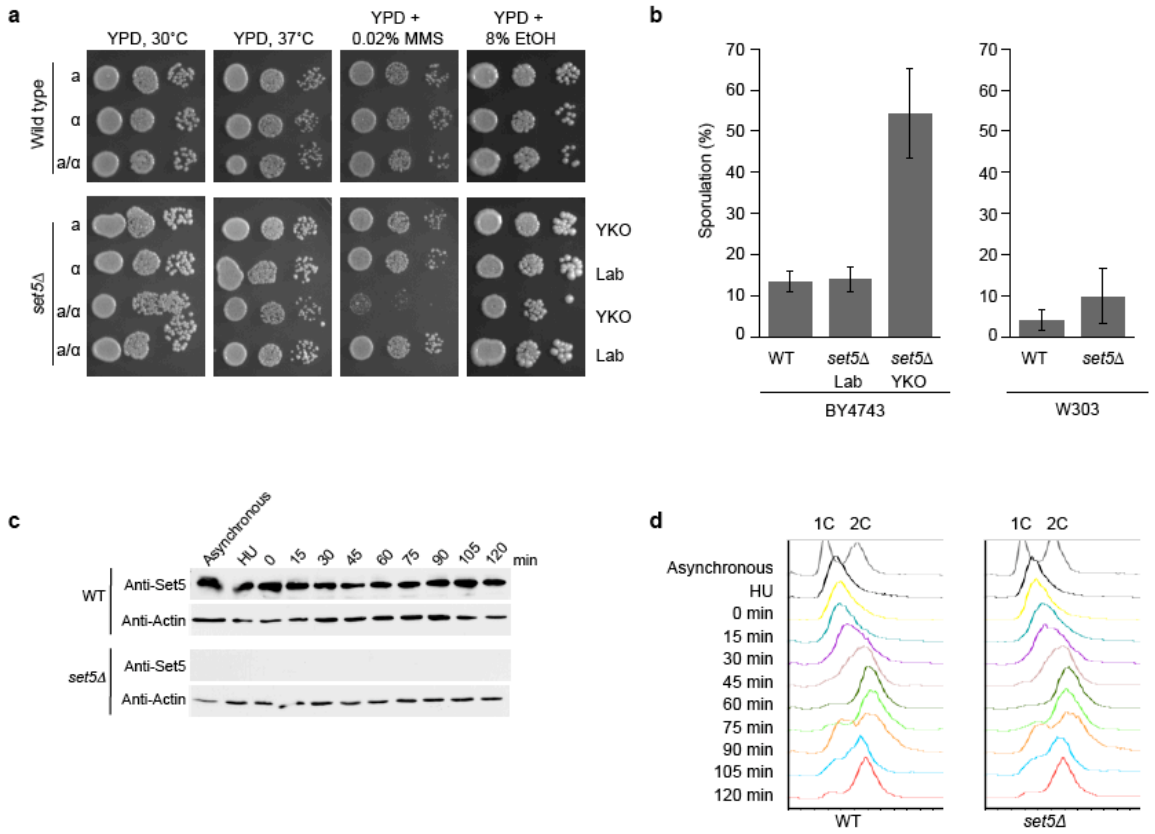


Supplementary Figure 2. Specificity of monomethyl K5 and K8 antibodies. The indicated biotinylated peptides were serially diluted two-fold from 100 ng to 3 ng and spotted on nitrocellulose. The blots were probed with rabbit anti-H4K5me1, rabbit anti-H4K8me1 or streptavidin-conjugated HRP as a loading control.



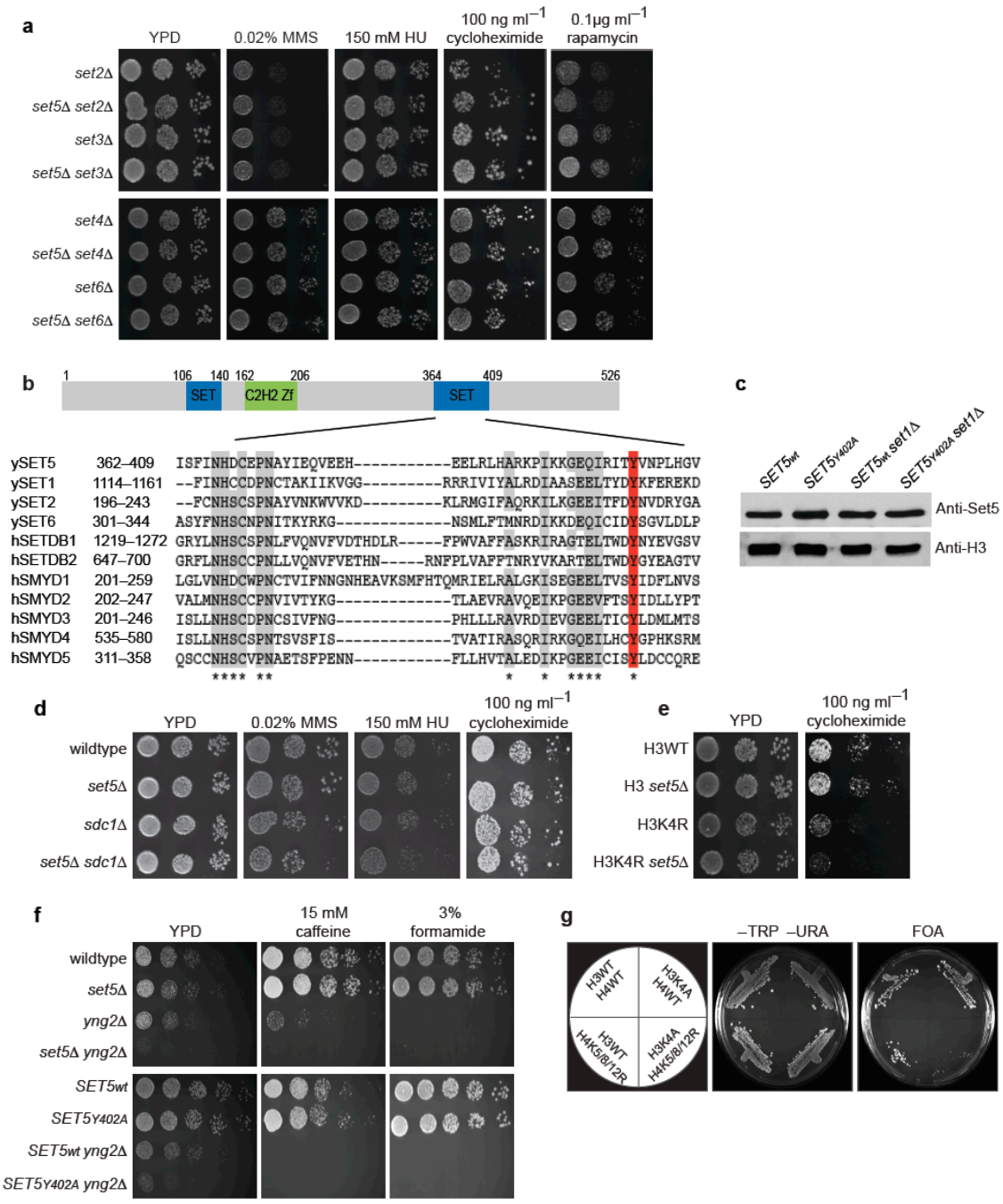
Supplementary Figure 3. Set5's activity and localization in cells. MS/MS spectrum of the precursor fragment at 782.960 m/z (see **Fig. 2b**) shows mostly H4K8me1, but lower levels of K5me1 (ion at 312 m/z; b2) and K12me1 (ion at

799 m/z) are also detected. Pr = propionyl amide chemical group from the derivatization. (**b**) Set5 localizes to the nucleus and the cytoplasm. Immunofluorescence of wild type and *SET5-MYC*-expressing cells using mouse anti-MYC antibody and DAPI (to stain DNA). Scale bar represents 5 μn. (**c**) Set5 is chromatin-associated. Whole-cell extracts (WCE) were fractionated into soluble (SUP) and chromatin fractions (PEL). Immunoblots with anti-Set5 and anti-H3 antibodies are shown.



Supplementary Figure 4. Phenotypic analysis of *set5Δ* cells.
(a) The *set5Δ* diploid (MATa/MATα) does not possess many of the phenotypes reported in the literature. Serial dilutions of both haploid and diploid wild type and *set5Δ* yeast from the Yeast Knockout (YKO) collection and strains generated independently in the lab (Lab) were spotted and grown in the indicated conditions. *set5Δ* diploid yeast were reported to be sensitive to these conditions based on experiments with the homozygous diploid YKO collection[1-4]. (**b**) Lab-generated *set5Δ* diploid strains sporulate similar to wild type, unlike the YKO homozygous diploid strain which shows increased sporulation efficiency. Wild type, lab-generated *set5Δ* (Lab) and YKO *set5Δ* (YKO) diploid strains from BY4743 background were analyzed for sporulation efficiency. Each strain was grown in YPD, transferred to YP + 2% KOAc and then transferred to sporulation media (1% KOAc, 0.1% yeast extract, 0.05% dextrose) at 30°C for 4 days. The percentage sporulation was determined by counting the number of tetrad asci formed (n>250 cells). Error bars represent the standard error of the mean (s.e.m) for at least three independent experiments. Similar rates of sporulation efficiency were observed in wild type and independently-generated *set5Δ* diploids from the W303 background (right graph). Several lines of evidence point to a possible chromosomal anomaly, including gene expression microarrays in which the commercial *set5Δ* diploid from the YKO collection differs from all other *set5Δ*

strains analyzed in that almost 50% of the genes on Chr. XIV are upregulated (data not shown). High rates of aneuploidy and spurious mutation in the YKO collection strains have previously been reported [5]. (**c**-**d**) Set5 is not required for cell-cycle progression under normal conditions. Wild type and *set5Δ* cells were arrested in S phase with 0.2 M HU for 3 hours and released into rich media. Samples were collected at the indicated timepoints and were prepared for (**c**) immunoblotting using the rabbit anti-Set5 antibody and a mouse anti-actin antibody as a loading control or (**d**) for flow cytometry analysis of the DNA content, as described[6]. DNA content was analyzed using FlowJo software.



Supplementary Figure 5. Analysis of genetic interactions between *SET5* and chromatin modifiers. (**a**) Phenotypic screen of double mutants combining *set5Δ* with *set2Δ, set3Δ, set4Δ, set5Δ* and *set6Δ*. Ten-fold serial dilutions of single and double mutants were spotted and grown at 30°C on the indicated media. Cells were grown for 2–5 days before imaging. (**b**) Mutation of tyrosine 402 to alanine (Y402A) renders Set5 catalytically inactive. Evolutionary conservation of the Set5 SET domain. Set5 contains a split SET domain (blue) and two consecutive C2H2 zinc fingers (green), one canonical and one unique[7]. Amino acids 362–409 of Set5 were aligned with the SET domains of other yeast and human SET

domain-containing enzymes using ClustalW. Conserved residues are highlighted in gray and indicated with an *. The invariant tyrosine (Set5 Y402) highlighted in red is implicated in SAM and target lysine binding[8]. (**c**) Protein expression levels of integrated wild type and Y402A *SET5* in the yeast strains used in **Figure 3c**. Immunoblotting of whole-cell extracts was performed with rabbit anti-Set5 and anti-H3 as a loading control. (**d**) The COMPASS subunit *SDC1* functionally interacts with *SET5*. (**e**) *set5*Δ yeast expressing the H3K4R mutation are impaired for growth in cycloheximide. (**f**) Growth of *set5*Δ *yng2*Δ and $SET5_{Y402A}$ *yng2*Δ double mutants on caffeine and formamide. (**g**) Plasmid shuffle showing a severe growth defect in cells expressing H3K4A and H4K5,8,12R histones.

Supplementary Tables

	KMT superfamily	Published substrates	Methylated lysine(s)	Extent of methylation	Ref.
Set1	SET	H3	Lys 4		
		Dam1	Lys 233	tri	9,10
Set2	SET	H3	Lys 36	tri	11
Set3	SET	unknown			
Set4	SET	unknown			
Set5	SET	unknown			
Set6	SET	unknown			
Rkm1	SET	Rpl23ab	Lys 105, Lys 109	di	12
Rkm2	SET	Rpl12ab	Lys 3	tri	13
Rkm3	SET	Rpl42ab	Lys 40	mono	14
Rkm4/Set7	SET	Rpl42ab	Lys 55	mono	14
		Cyc1			
Ctm1	SET	(cytochrome c)	Lys 72	tri	15
Efm1	SET	eEF1A	n.d.	mono	16
Rkm5	Seven β-strand	Rpl1ab	Lys 46	mono	17
See1	Seven β-strand	eEF1A	n.d.	di	16
Dot1	Seven β-strand	H3	Lys 79	tri	18,19

Supplementary Table 1. Yeast SET domain and seven β-strand lysine methyltransferases (KMTs) with known substrates and target sites. Set1, Set2 and Dot1 are known to target histone H3, whereas Rkm1–5, Ctm1, Efm1 and See1 target non-histone proteins. Substrates for Set3 through Set6 remain to be identified.

Strain name	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	YKO
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	YKO
BY4743	*MATa/α his3Δ1/ his3Δ1 leu2Δ0/ leu2Δ0 lys2Δ0/ lys2Δ0 ura3Δ0/ ura3Δ0*	YKO
YGM6	*MATa set5Δ::KANMX*	YKO
YGM144	*MATα set5Δ::KANMX*	This study
YGM82	*MATa/α set5Δ::KANMX/ set5Δ::KANMX*	YKO
YGM136	*MATa/α set5Δ::KANMX/ set5Δ::KANMX*	This study
YEG048	W303; *MATa/α RAD5/RAD5 leu2-3,112/leu2-3,112, trp1-1/trp1-1 can1-100/can1-100 ura3-1/ura3-1 ade2-1/ade2-1 his3-11,15/his3-11,15*	This study
YEG049	W303; *MATa/α set5Δ::KANMX/ set5Δ::KANMX*	This study
YEG022	*MATa SET5::SET5-MYC::HIS3MX*	This study
YGM76	*MATa set5Δ::NATMX*	This study
YGM2	*MATa set1Δ::KANMX*	YKO
YGM77	*MATa set5Δ::NATMX set1Δ::KAN MX*	This study
YGM3	*MATa set2Δ::KANMX*	YKO
YGM149	*MATa set5Δ::NATMX set2Δ::KANMX*	This study
YGM4	*MATa set3Δ::KANMX*	YKO
YGM150	*MATa set5Δ::NATMX set3Δ::KANMX*	This study
YGM5	*MATa set4Δ::KANMX*	YKO
YGM151	*MATa set5Δ::NATMX set4Δ::KANMX*	This study
YEG095	*MATa set6Δ::KANMX*	This study
YGM152	*MATa set5Δ::NATMX set6Δ::KANMX*	This study
YEG086	*MATa hir1Δ::HIS3MX*	This study
YEG087	*MATa set5Δ::KANMX hir1Δ::HIS3MX*	This study
YGM167	*MATa SET5::SET5$_{wt}$::NATMX*	This study
YGM168	*MATa SET5::SET5$_{Y402A}$::NATMX*	This study
YGM169	*MATa SET5::SET5$_{wt}$::NATMX set1Δ::KANMX*	This study
YGM170	*MATa SET5::SET5$_{Y402A}$::NATMX set1Δ::KANMX*	This study
YEG097	*MATa sdc1Δ::KANMX*	YKO
YEG113	*MATa set5Δ::HIS3MX6*	This study
YEG115	*MATa set5Δ:: HIS3MX6 sdc1Δ::KANMX*	This study
YGM78	*MATa yng2Δ::KANMX*	This study
YGM79	*MATa set5Δ::NATMX yng2Δ::KANMX*	This study
YGM178	*MATa SET5::SET5$_{wt}$::NATMX yng2Δ::KanMX*	This study
YGM179	*MATa SET5::SET5$_{Y402A}$::NATMX yng2Δ::KanMX*	This study
WZY42	*MAT a, ura3–52, lys2–801, ade2–101, trp1Δ63, his3Δ200, leu2Δ1, hht1-hhtf1::LEU2, hht2-hht2::HIS*), YCp50-copyII (*ura3^{+},HHT2-HHF2)*	9
YGM25	As WZY42; *pRS314-HHT2-HHF2*	9
YGM27	As WZY42; *pRS314-HHT2 K4R-HHF2*	9
YEG119	As WZY42; *set5Δ::KANMX pRS314-HHT2-HHF2*	This study
YEG120	As WZY42; *set5Δ::KANMX pRS314-HHT2 K4R-HHF2*	This study

Supplementary Table 2. Yeast strains used in this study. All strains are derived from BY4741 obtained from the Yeast Knockout Collection (Open Biosystems) unless otherwise indicated.

Methods

Yeast strains, plasmids and reagents

BY4741 wild type and *set5*Δ haploid strains and BY4743 wild type and *set5*Δ diploid strains were obtained from the Yeast Knockout Collection (Open Biosystems). Other deletion strains were independently generated by standard PCR-based methods using a kanamycin resistance deletion cassette. *SET5* was genomically tagged at the C terminus in wild type cells with a sequence encoding 9-MYC. The $SET5_{Y402A}$ and corresponding $SET5_{WT}$ strains were generated by integration of a cassette originating from *pFA6a-SET5Cterm::KANMX*, a plasmid containing either the wild type or Y402A C-terminal region of *SET5* followed by the *KANMX* selection marker. Integrants were confirmed by sequencing.

To generate plasmid shuffle strains, standard PCR-based site-directed mutagenesis on the plasmid pRS314-*HHT2-HHF2*[20] (kindly provided by S. Briggs, Purdue University) was used to mutate H3 lysine 4 to alanine (H3K4A), and H4 lysines 5, 8 12 to arginine (H4K5,8,12R). Mutations were verified by sequencing. Constructs were then transformed into strain WZY42 (S288C derivative; kindly provided by S. Dent, UT MD Anderson Cancer Center), carrying YCp50-copyII (*ura3*$^{+}$,*HHT2-HHF2)*[9], and selected on 5-FOA-containing plates for cells that lost the wild type copy of *HHT2-HH*F2.

All glutathione *S*-transferase (GST) expression constructs were cloned into and expressed from *pGEX-6P-1* (GE Healthcare), including full length SET-domain-containing proteins (Set2 to Set6) and the N-terminal tails of histone H3 (amino acids 1–45), H4 (1–31), H2A (1–27) and H2B (1–37). Other substrates used in methylation assays include recombinant histone H4 (Upstate) and calf thymus histones (Worthington). The mutant H4 tail library was generated by cloning the H4 N terminus (amino acids 1–31) with all the lysines replaced by arginines into *pGEX-6P-1*. Each arginine was individually restored back to lysine by site-directed mutagenesis. Plasmids *pGEX-6P-1-H4tail* and *pGEX-6P-1-H2Atail* were

subjected to site-directed mutagenesis to mutate lysines individually or in combination to arginine. Site-directed mutagenesis of the *pGEX-6P-1-SET5*$_{WT}$ plasmid was used to generate *pGEX-6P-1-SET5*$_{Y402A}$. All sequences were confirmed by DNA sequencing.

The antibodies used in this study are anti-H3 (ab1791; Abcam), anti-H4 (ab7311; Abcam), anti-H3K4me1 (ab8895; Abcam), anti-H3K36me1 (ab9048; Abcam), anti-H3K79me1 (ab2886; Abcam), anti-myc 9E10 (Abcam) and anti-actin Ab-5 (612656, BD Biosciences). Anti-H4K5me1 and anti-H4K8me1 rabbit polyclonal antibodies were raised using peptides as antigen (Abmart). The rabbit polyclonal anti-Set5 antibody was generated (Covance) against full-length Set5 purified and cleaved from GST. Biotinylated H4 and H3 unmodified and modified peptides were synthesized at the Yale W.M. Keck facility as previously described[21]. Antibody specificity was tested using peptide dot blots, as described[22].

Methylation assays

Methylation assays were performed using 5 μg of GST-purified enzyme with 1 μg of substrate, in a buffer containing 50 mM Tris-HCl (pH 8.0), 10% glycerol, 20 mM KCl, 5 mM $MgCl_2$ and 1 mM PMSF. As methyl-group donors, 0.3 mM *S*-adenosyl-methionine (SAM, Sigma) or 2 μCi ^{3}H-SAM (Amersham) were added to the reactions and incubated at 30°C overnight. Proteins were resolved by SDS-PAGE, followed by autoradiography, immunoblot analysis or Coomassie staining (GelCode Blue stain reagent, Thermo Scientific).

Histone acid extraction

Yeast core histones were purified as previously described[23], starting with 1 L of log-phase cells. Histones were either resolved by SDS-PAGE and immunoblotted (40 μg protein loaded for anti-H4K5me1 and anti-H4K8me1 blots, 4 μg for anti-H4 blot and 20 μg for anti-H3 blot) or subjected to mass spectrometry analysis.

Quantitative mass spectrometry

In vivo-derived samples were analyzed as previously described[24]. In brief, histone samples from the *set5*Δ strain were modified by D5-propionylation and wild type samples by D0-propionylation. Following trypsin digestion, samples were desalted before loading onto a C18 packed 75 µm fused silica capillary column with ESI tip by an autosampler (AS-2; Eksigent Technologies Inc.). Peptides were introduced into an Orbitrap mass spectrometer and separated by RP-HPLC gradient (1–100% buffer B in 110 mL; buffer A = 0.1 M acetic acid, buffer B = 70% acetonitrile in 0.1 M acetic acid) by an HPLC pump (1200 series; Agilent). The Orbitrap mass spectrometer was operated in data-dependent MS/MS mode, obtaining a full MS at 30000 resolution and 7 MS/MS spectra in the ion trap. All MS/MS spectra were manually inspected. For the analysis of Set5's activity on peptides, an H4-biotinylated peptide (amino acids 1–23) was separated by a reversed phase gradient from 1–100% buffer B in 12 minutes. For relative yield the MS1 was added across both nearly co-eluting peaks. Data-dependent electron transfer dissociation (ETD) was performed with a reaction time of 80 ms. The identity of H4 was thereby confirmed by MS2 (data not shown), but not the identity of the putative methylated species. The 7th charge state of the monomethylated H4 peak was subsequently targeted for ETD MS2 by exclusively selecting 733 m/z and adding the MS2 across the peak. All ETD MS/MS data were analyzed manually.

Chromatin fractionation

Yeast cells were grown to mid-log phase in 100 mL YPD, harvested and frozen in liquid nitrogen. Cell pellets were processed for chromatin fractionation as previously described[25-27]. Collected fractions were boiled in SDS loading buffer and equivalent amounts were subjected to SDS-PAGE. Immunoblots were probed with rabbit anti-Set5 antibody (1:3000; Covance) and rabbit anti-H3 antibody (1:10,000; Abcam).

Co-immunoprecipitation

200 ml of log-phase yeast were harvested and frozen in liquid nitrogen. Pellets were resuspended in 800 μl lysis buffer (50 mM Hepes-KOH pH7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml aprotinin) and whole-cell extracts were obtained by bead-beating (Vortex Genie2, Scientific Industries) followed by sonication (Branson Digital Sonifier) using standard procedures. Immunoprecipitation reactions were performed by incubating 5 mg of extract with 5 μg of anti-H3 antibody (Abcam) overnight at 4ºC. Protein A/G beads (Ultralink Resin, Thermo Scientific) were added to the samples and incubated for 2 hr at 4ºC. Beads were washed 2 times in lysis buffer, 2 times in lysis buffer with 300 mM NaCl, and 2 times in TE. Elution was performed by addition of 50 μl SDS loading buffer and boiling for 10 min. Proteins were resolved by SDS-PAGE and immunoblotted.

Immunofluorescence

Indirect immunofluorescence was performed on spheroplasted yeast cells as described previously[28]. The 9E10 mouse anti-MYC antibody (Abcam) was used at a dilution of 1:250; AlexaFluor$_{555}$-conjugated goat anti-mouse secondary antibody (Invitrogen) was used at a dilution of 1:1000. DNA was stained using ProLong Gold antifade reagent with DAPI (Invitrogen). Microscopy was performed on a Leica DM5000B equipped with a Qimaging RETIGA Exi camera controlled by QCapture Pro software.

Supplementary References

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