Supplementary Information

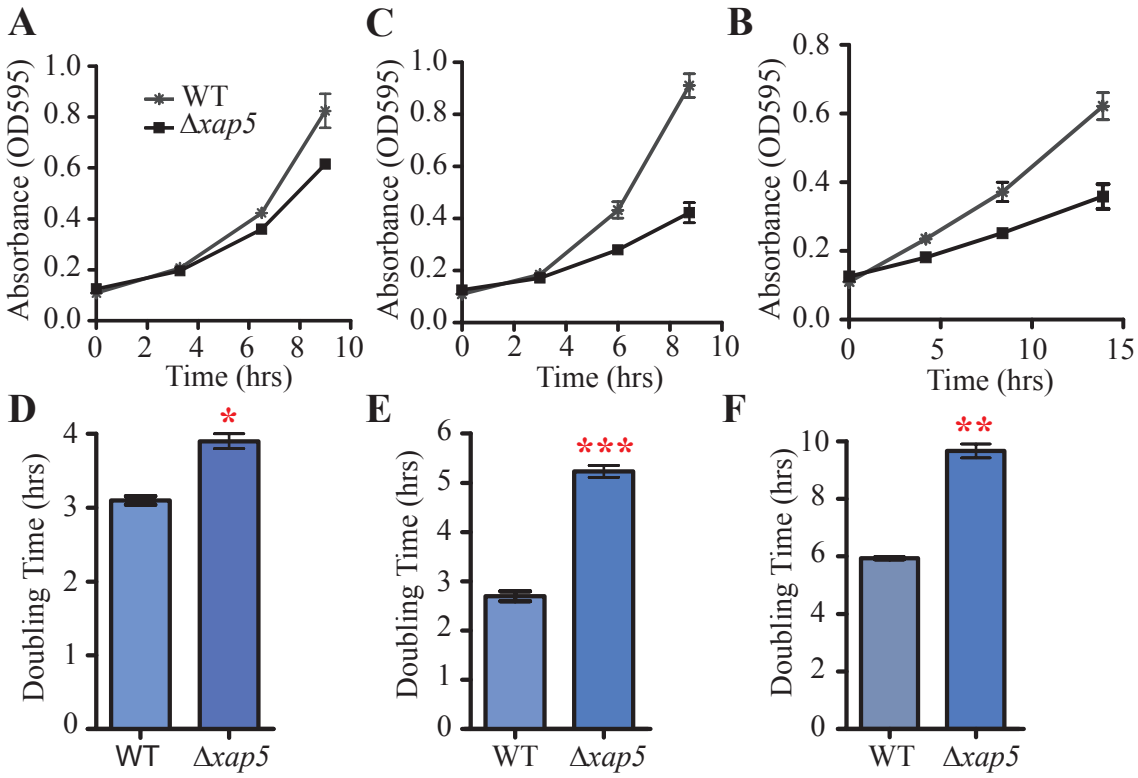


Fig S1: *S. pombe* mutants have a slow growth phenotype. Growth curves for *S. pombe* *Δxap5* mutants grown in EMM50 at 30°C (A, D), at 37 °C (B, E) and at 21 °C (C, F). Mean ± s.e.m. of at least three independent experiments with at least three replicates (n≥3). Asterisks denote statistical significance (D-F, Student's t-test, * indicates p=0.0023, ** indicates p=0.0001, *** indicates p<0.0001).

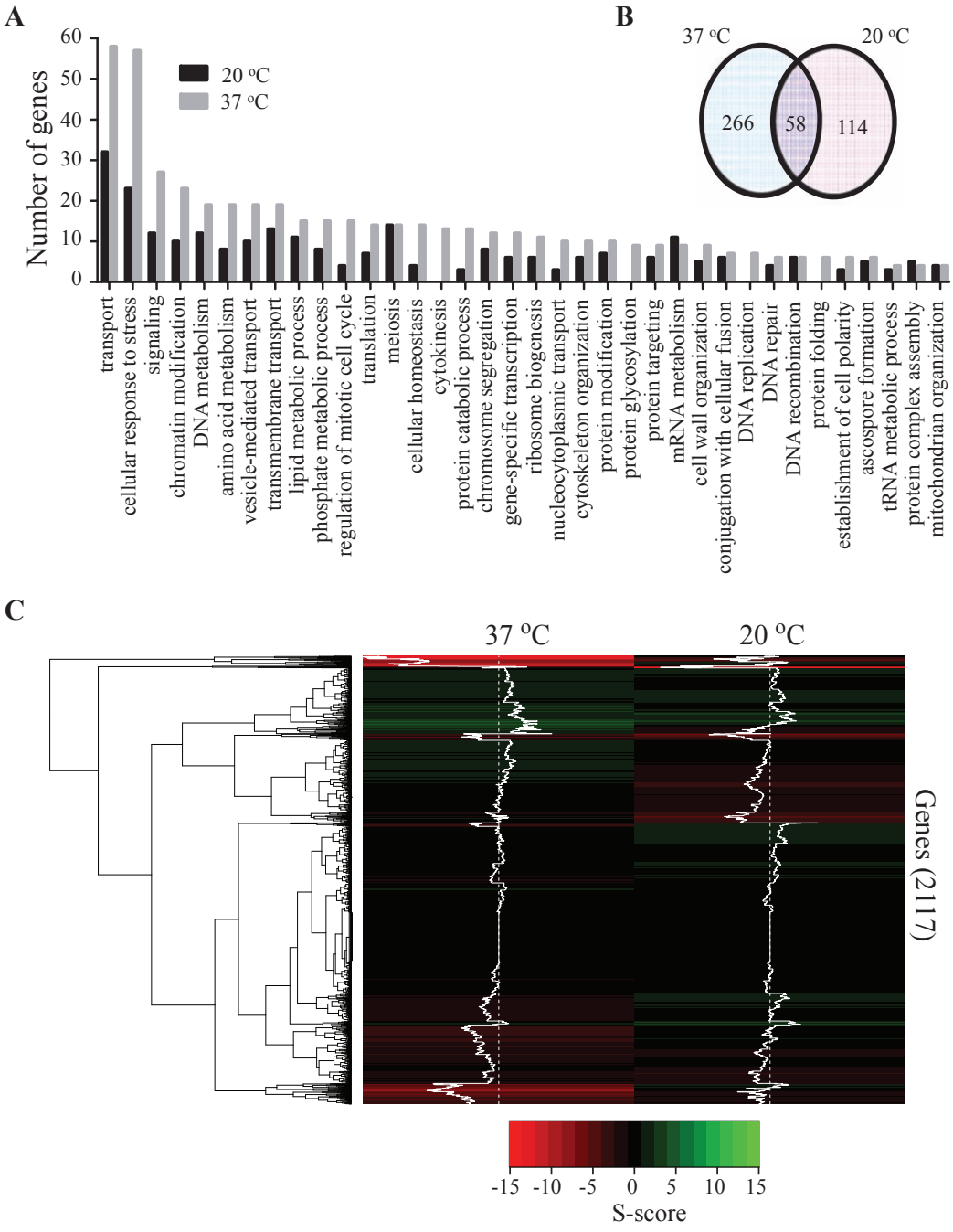


Fig S2: *xap5* exhibits similar genetic interactions at 37 °C and 20 °C (A) Gene ontology category distribution for biological function of the genes significantly genetically interacting with *xap5* at 37 °C and 20 °C. (B) Number of genes significantly interacting with *xap5* at 37 °C and 20 °C and the overlap (degree of overlap is greater than expected by chance; Fisher's exact test, p<2.2e-16). (C) Heatmap of the S-score profiles of all genetic interactions. S-scores of individual genes are indicated with continuous white lines while dotted white lines mark the origins (0) of the X-axes.

Table S1: Significantly enriched GO categories for biological function of genes with genetic interactions positively correlated with *xap5* at 37 °C (p≤0.05)

GO ID	Description	p-value	Corrected p-value	Sample frequency	Background frequency
6338	chromatin remodeling	1.7772E-7	1.4288E-4	28/133, 21%	71/869, 8.2%
16568	chromatin modification	4.7574E-7	1.9125E-4	32/133, 24%	91/869, 10.5%
6325	chromatin organization	1.4478E-5	3.8801-3	32/133 24.06%	104/869, 12%
6476	protein amino acid deacetylation	3.6422E-5	5.8567E-3	9/133, 6.8%	14/869, 1.6%
16575	histone deacetylation	3.6422E-5	5.8567E-3	9/133, 6.8%	14/869, 1.6%
16197	endosome transport	7.8729E-5	8.3255E-3	5/133, 3.8%	5/869, 0.6%
16570	histone modification	8.2841E-5	8.3255E-3	19/133, 14.3%	52/869, 6%
16569	covalent chromatin modification	8.2841E-5	8.3255E-3	19/133, 14.3%	52/869, 6%
16043	cellular component organization	1.0880E-4	9.7197E-3	69/133, 51.9%	322/869, 37%
42147	retrograde transport, endosome to Golgi	5.2791E-4	4.2444E-2	4/133, 3%	4/869, 0.46%

Table S2: Multiple members of important chromatin remodeling complexes are among genes with genetic interactions correlated with *xap5* at 37 °C and 20 °C

Complex	Sample frequency 20°C	Sample frequency 37°C	Background frequency	Genes
Set1C/COMPASS complex	7/119 (5.9%)	5/133 (3.8%)	8/869 (0.9%)	*set1, swd1, swd2, swd3, spf1, ash2, sdc1*
Rpd3L-Expanded complex	4/119 (3.4%)	5/133 (3.8%)	8/869 (0.9%)	*rxt2, hif2, prw1, hos2, set3*
Set3 complex	3/119 (2.5%)	3/133 (2.3%)	3/869 (0.3%)	*hif2, hos2, set3*
Swr1 complex	3/119 (2.5%)	4/133 (3%)	7/869 (0.8%)	*swr1, msc1, swc5, pht1*

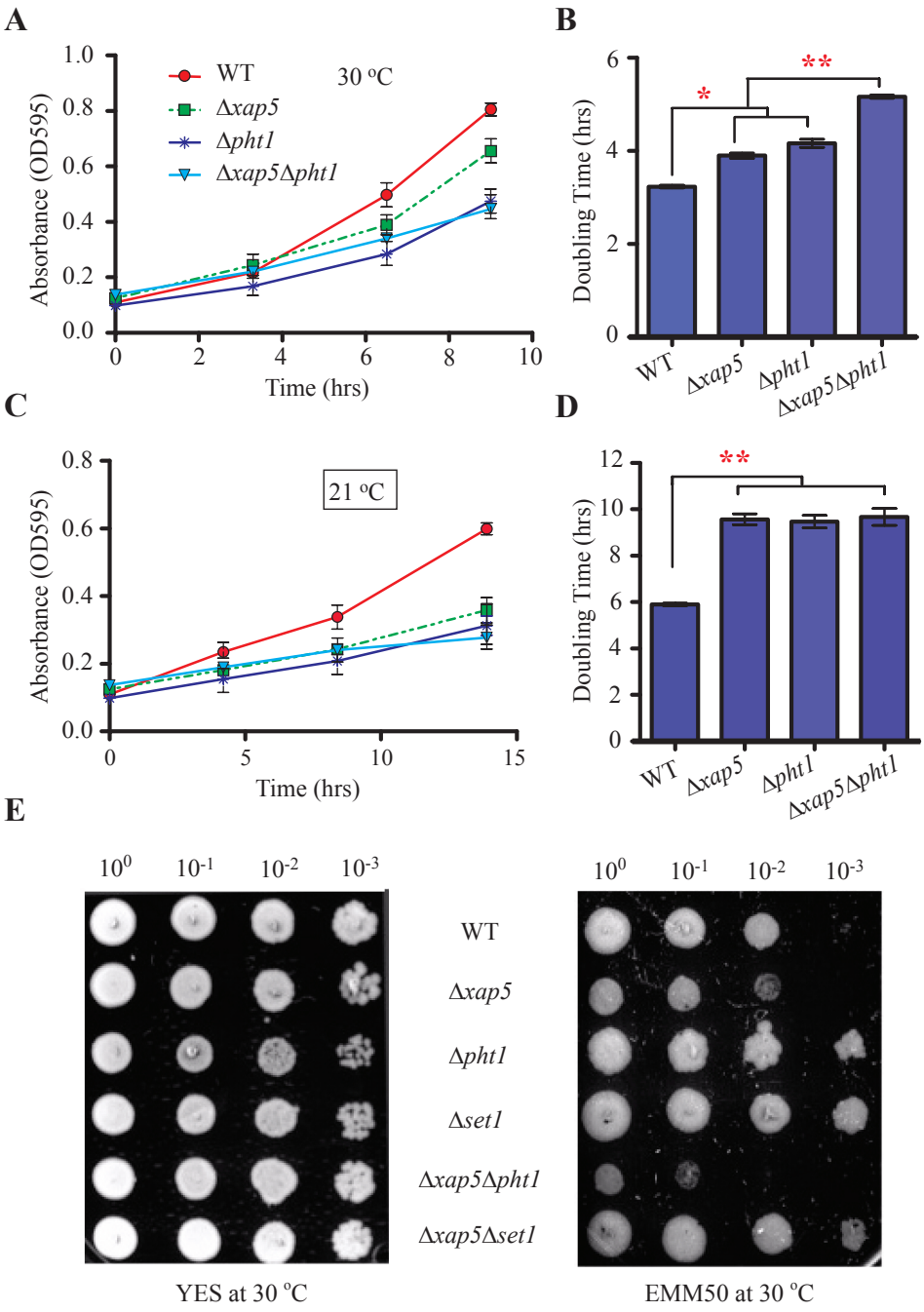


Fig S3: The *S. pombe* H2A.Z ortholog *pht1* and H3K4 methyltransferase *set1* genetically interact with *xap5*. Growth curves for *S. pombe Δxap5, Δpht1 and Δxap5Δpht1* mutants grown in EMM50 at 30 °C (A, B) and 21 °C (C, D). *xap5* has opposite genetic interactions with *pht1* and *set1*. Mean ± s.e.m. of three independent experiments with three replicates (n≥3). Asterisks denote statistical significance (one-way ANOVA with Bonferroni's multiple comparison test, * indicates p=0.0006, ** indicates p<0.0001). (E) Comparison of growth of *Δset1* and *Δxap5Δset1* mutants at 30 °C in YES and EMM50 with that of *Δxap5*, *Δpht1* and *Δxap5Δpht1* mutants.

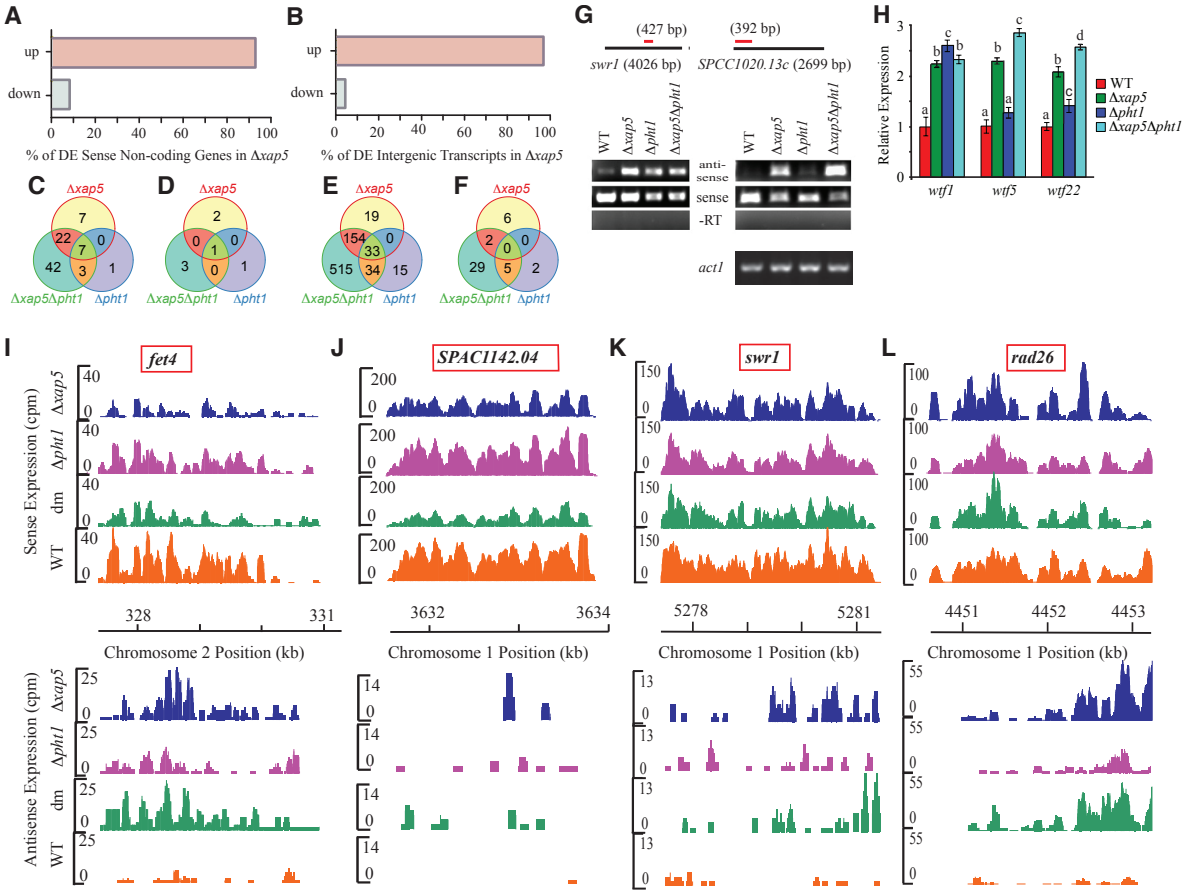


Fig S4: Many types of aberrant transcripts are upregulated in *Δxap5*. Percentage of (A) sense non-coding transcripts (SNC) and (B) intergenic transcripts that are up- and down-regulated in Δ*xap5*. Venn diagrams showing the overlap between (C) up- and (D) down-regulated SNC transcripts and (E) up- and (F) down-regulated intergenic transcripts in the indicated mutants. Transposon-associated and antisense (AS) sequences are upregulated in Δ*xap5*. (G) Strand-specific RT-PCR confirmation of upregulation of AS transcripts at representative loci; representative of two independent experiments (H) Quantitative RT-PCR confirmation of upregulation of *wtf* loci. Lowercase letters denote statistically significantly different groups (one-way ANOVA with Bonferroni's multiple comparison test, $p<0.05$); the values are the mean±s.e.m of three independent experiments, each with three biological replicates (n=3). Abundance of sense and antisense transcripts are not correlated in Δ*xap5*. (I-L) Representative loci with different patterns of regulation of sense and AS transcripts. In 41% of the loci with significantly upregulated AS transcripts, sense transcripts are significantly ($p\leq0.01$) downregulated, as shown for the (I) *fet4* and (J) *SPAC1142.04* loci. 21% of the loci with upregulated AS transcripts do not show significant alterations in levels of sense transcripts, as shown for the (K) *swr1* and (L) *rad26* loci. dm = Δ*xap5*Δ*pht1* double mutant. Statistical

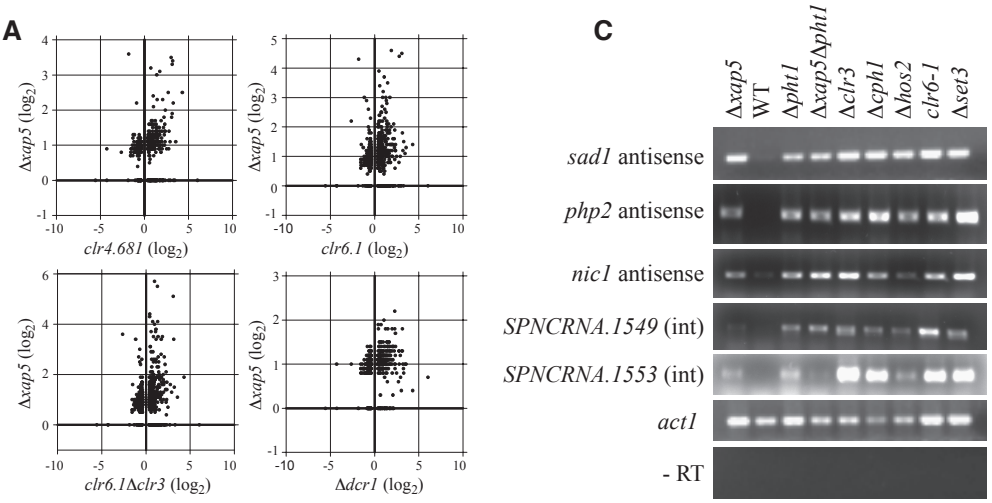
significance of differential expression was determined using the negative binomial exact test ($p \leq 0.01$, fold change ≥ 2). Panels A-F and I-L are based on three biological replicates.

Table S3. GO-term enrichment for biological process ($p \leq 0.05$) for genes upregulated in the Δ*xap5* mutant

GO-ID	Description	Corrected p-value	Sample (%)	Genome (%)
33554	Cellular response to stress	6.31E-74	41.58	13.10
9408	Response to heat	3.22E-08	3.26	0.63
6979	Response to oxidative stress	4.00E-07	4.47	1.26
61077	Chaperone-mediated protein folding	6.88E-03	1.20	0.23
34614	Cellular response to reactive oxygen species	1.29E-02	1.55	0.40
51039	Positive regulation of transcription, meiotic	1.49E-02	1.03	0.19
5991	Trehalose metabolic process	1.59E-02	0.86	0.13
90329	Regulation of DNA-dependent DNA replication	2.20E-02	2.06	0.71
45014	Negative regulation of transcription by glucose	2.43E-02	1.03	0.21
42542	Response to H_2O_2	4.15E-02	1.03	0.23

Table S4. GO-term enrichment for biological process (p≤0.05) for genes downregulated in the Δ*xap5* mutant

GO-ID	Description	Corrected p-value	Sample (%)	Genome (%)
42254	Ribosome biogenesis	4.86E-27	19.48	6.14
34660	ncRNA metabolic process	2.93E-26	18.16	5.56
34470	ncRNA processing	3.40E-19	14.61	4.78
6364	rRNA processing	3.86E-18	11.61	3.35
45948	Positive regulation of translational initiation	9.03E-10	3.75	0.69
6396	RNA processing	4.53E-09	16.10	8.16
43039	tRNA aminoacylation	1.34E-08	3.56	0.71
10467	Gene expression	2.17E-07	34.08	23.77
10608	Posttranscriptional regulation of gene expression	2.92E-06	5.99	2.26
46148	Pigment biosynthetic process	2.67E-04	1.87	0.40
42255	Ribosome assembly	1.06E-03	1.69	0.38
51169	Nuclear transport	3.83E-03	4.68	2.26
6913	Nucleocytoplasmic transport	3.83E-03	4.68	2.26
6360	Transcription from RNA polymerase I promoter	6.25E-03	1.87	0.57
9451	RNA modification	1.22E-02	3.37	1.55
30488	tRNA methylation	1.46E-02	1.31	0.34
1510	RNA methylation	1.46E-02	1.69	0.54
31291	Ran protein signal transduction	3.81E-02	0.56	0.08
6402	mRNA catabolic process	3.93E-02	2.43	1.09
956	Nuclear-transcribed mRNA catabolic process	4.40E-02	1.69	0.63



B

Spearman Correlation Coefficients

	clr4.681	*clr6.1*	*clr6.1Δclr3*	*Δdcr1*
Δ***xap5***	0.31495	0.2273378	0.3244858	0.2585436
Δ***xap5*** **p-values**	p<2.2e-16	p=6.49e-11	p<2.2e-16	p=1.077e-13
Δ***xap5Δpht1***	0.2855117	0.2477814	0.3157752	0.2226202
Δ***pht1***	0.1811902	0.08298737	0.19621192	0.2196517

Fig S5: Correlation between gene expression in Δ*xap5* and mutants with lesions in known silencing and RNAi pathway genes. (A) Transcriptome profile of *Δxap5* compared to those of known silencing mutants *clr4.681, clr6.1, clr6.1*Δ*clr3* and Δ*dcr1* (the latter data are from Hansen et al. [1]). (B) Spearman correlation coefficients based on a genome-wide comparison of transcripts between the specified mutant pairs. (C) Expression levels of selected antisense and intergenic (int) loci in single and double mutant *Δxap5* and *Δpht1* strains compared to strains with lesions in genes encoding components of several different histone deacetylase complexes. The gels shown in panel C are representative of two independent experiments.

Table S5. Mean normalized read counts (mean±SD) of the genes shown in Fig.4

Gene	Mean Δ*xap5*	Mean Δ*pht1*	Mean WT
SPAPB15E9.06	6.0±1.6	4.3±1.4	1.9±0.3
SPAC27E2.01	39.5±2.4	40±1.31	33.4±0.4
Tf2-5	159±15.9	176.4±13.7	107.2±33.6
wtf8	12.2±2.7	9.5±1.5	6.3±1.6
SPCC306.11	90.6±13.5	50.9±12.5	18.6±6.3
SPCC306.06c	151±16.2	155.7±27.3	149.8±33.6
SPCC306.07c	36.6±3.2	54.6±3.96	47.2±3.8
SPCC306.08c	230.9±19.8	124.9±10.3	110.7±14.6
cap1	316.8±3.6	279.3±17.2	274±8.2
alp16	106.4±5	102.3±12.6	91.5±10
SPCC4G3.18	169.4±7.1	227.5±24.7	209.6±18.1

Online Methods

Growth Analysis and Gene Transformations in *S. pombe*

Strains used are listed below. All yeast media and standard methods were used as described by Forsburg and Rhind [2]. Doubling time was calculated using $DT=\log(2^{t2-t1})/\log(A_{t2}/A_{t1})$, where A=cell density (as measured by OD595) and t=time. Gateway entry vector, pENTR-D-TOPO (Invitrogen) and the destination vector pDUAL-FFH1c [3] with a thiamine repressible yeast *nmt1* promoter were used to express the plant *XCT* cDNA or *xap5* gDNA in the *Δxap5* mutant background.

Yeast strains used in the study

Strain	Description
Δ*xap5*	(Bioneer-BG4186) h^+, ade6-M216, ura4-D18, leu1-32, SPCC1020.12c::KanMx4
WT	(ED668) h^+, ade6-M216, ura4-D18, leu1-32
Δ*pht1*	h^-, ade6-M216, ura4-D18, leu1-32, SPBC11B10.10c::NatMx4
Δ*set1*	h^-, ade6-M216, ura4-D18, leu1-32, SPCC306.04c::NatMx4
Δ*xap5*Δ*pht1*	h^-, ade6-M216, ura4-D18, leu1-32, SPCC1020.12c::KanMx4, SPBC11B10.10c::NatMx4
Δ*xap5*Δ*set1*	h^-, ade6-M216, ura4-D18, leu1-32, SPCC1020.12c::KanMx4, SPCC306.04c::NatMx4

Genetic Interaction Analysis

For identifying genetically interacting partners, the epistatic miniarray profile (E-MAP) method was used. In E-MAP, a mutant strain (Δ*xap5*) with one marker is crossed to a library of yeast deletion strains (consisting of the 2117 mutants that passed quality control analysis of the original total library of 2662 mutants [4]) carrying a second marker to systematically recover haploid double mutant strains. Sizes of colonies of double and single mutant strains grown for a defined period of time after transfer of a defined number of cells are then measured in a high-throughput manner. From these growth rate data, interaction scores (S-scores) were determined [4, 5]. The S-score (which is a modified t-value) is a measure of the genetic interaction between each tested gene and *xap5*. Cells were grown at 20 ºC, 30 ºC or 37 ºC in YES media. $-2.5 \leq$ S-score$\geq +2$ were used as cutoffs for statistically significant genetic interactions. GO category enrichment ($p \leq 0.05$) analyses were performed with BiNGO 2.44 [6] (a Cytoscape plugin), employing a hypergeometric test with subsequent Benjamini and Hochberg false discovery rate corrections.

RNA-seq Analysis

Exponentially growing cells at 37 ºC in EMM50 were used for RNA extraction with three replicates per each genotype. Cell lysis was performed as recommended by Sabatinos and Forsburg [7] in 1 ml Trizol reagent (Invitrogen). RNA was extracted as per the Invitrogen Trizol protocol. Extracted RNA was digested with DNase I (Qiagen) for 30 minutes at room temperature and cleaned up with 3M sodium acetate. rRNA was removed using the Ribo-Zero rRNA removal kit (human/mouse/rat, Epicentre). Strand specific cDNA libraries were made with random primers using an optimized version of the protocol described by Wang et al. [8], a protocol that doesn't use actinomycin D. Samples were multiplexed and 100 bp single reads were sequenced in an Illumina Hi-seq sequencer in two lanes with an average sequence coverage of 11.8 million reads/library. All quality control steps were performed using the fastx_tool kit (http://hannonlab.cshl.edu/ fastx

toolkit/index.html). Reads were mapped to the complete *S. pombe* cDNA and genomic DNA (EF2.12, ftp://ftp.ensemblgenomes.org/ pub/release12/fungi/fasta/schizosaccharomyces_pombe/) using BWA, ver. 0.5.8c [9]. Differential expression analysis was performed using edgeR [10] where statistical significance of differential expression is determined using the negative binomial exact test. Data were visualized with IGB-6.2.2 [11] and R (ver. 2.14.0). BedTools [12] were used for analysis of reads corresponding to intergenic and LTR loci.

RT-PCR Analysis

RNA was extracted as for RNA-seq analysis. Randomly primed cDNA prepared with SuperScript II Reverse transcriptase (Invitrogen) was used for quantitative RT-PCR. The reactions were performed using an iCycler (Bio-Rad) in 40 mM Tris HCl pH 8.4, 100 mM KCl, 6 mM $MgCl_2$, 8% glycerol, 20 nM fluorescein, 0.4X SYBR Green I (Molecular Probes), 1xBSA (New England Biolabs), 1.6 mM dNTPs, 2.5 μM of each primer, and 5% diluted cDNA along with *Taq* polymerase. Samples were run in triplicates and relative starting quantity was estimated using the $\Delta\Delta C_T$ [13] method. Data presented were normalized to *act1* expression levels. Melt curve analysis was performed following amplification to confirm specificity of products over primer dimers. Primers used for *act1* were described in Helmlinger D*, et al.* [14]. Strand-specific RT-PCR reactions were performed with Qiagen one-step RT-PCR kit as per the manufacturer's protocol. All primers used are listed in below.

Primers used in the study

Purpose	Primers
*swr1*_antisense_RT-PCR (427 bp)	5'TCCTTCAAATTAGCAAAAGCAGA3' 5'GGGGCCCTCATTTAATTATTGTA3'
*SPCC1020.13c*_antisense_RT-PCR (392 bp)	5'GCTCATTGTATGAAGAATTCAAAA3' 5'AATCTATTGCTATTTTTCCAAAGC3'
Tf-2 retrotransposons_sense_qRT-PCR	5'CGCTCTCTCAAATGAACAAAGTT3' 5'CCTTTTATTGGCTTTGGTAGCTT3'
*wtf1*_sense_qRT-PCR	5'TCATACCTCAGGTCGGATAAAAA3' 5'TACTTGGAATTTGGTCTTGGTGT3'
*wtf5*_sense_qRT-PCR	5'GGAAAAGGAATCAAGCACTTTTT3' 5'CTTGGTGATCCGACAAGTATGTT3'
*wtf22*_sense_qRT-PCR	5'TTATGGCAAAGATGAATGGGTAT3' 5'ATCCAAGTTTCATAGAAGCACCA3'
r*ps2402*-3'end_*cnt5*-3'end_readthrough_antisense _RT-PCR (568 bp) – from Zofall et al., [15]	5'CGCAAGCAACGTAAGAACAGAG3' 5'TATCAAAGGGCTGTTGTAAAGGC3'
*sad1*_antisense_RT-PCR	5'TCATCAGATAATTCCCAACCATC3' 5'TAATTGTCCCTCTGAACCTTGAA3'
*php2*_antisense_RT-PCR	5'GTCTGAGTCTCCAGCTCCTATCA3' 5'GGGATATGTTTGCTGACCAATTA3'
*nic1*_antisense_RT-PCR	5'AAGTTTGCAGATCGATGGAATAA3' 5'AATGCTGCTATAACCGAAACAAA3'
*SPNCRNA.1549*_sense (intergenic)_RT-PCR	5'AAAATTCCAAGGAAGAAAAGTCG3' 5'GCCTGTGTCCTATATCTTGTTGC3'
*SPNCRNA.1553*_sense (intergenic)_RT-PCR	5'ACCTTTCGTTAAGAGGCGTAAAC3' 5' AAAATACCCATATCGTCCTCGTT3'

Antisense Read Comparison: TSS vs. TTS (Fig. 2K)

Protein coding gene coordinates (gene starts and gene ends) of *S. pombe* were extracted from http://fungi.ensembl.org/biomart/martview/ (5117 genes). Overlapping genes (negative values for intergenic regions) were removed leaving 3632 protein coding genes. Genes with low expression levels (defined as those that did not have at least one read per one million counts in three or more of our samples) were also discarded, leaving 2057 protein coding genes in our analysis. Antisense reads for upstream of transcription start site (TSS) and downstream of transcription termination site (TTS) were extracted for up to 500 bp in 100 bp bins. A similar strategy was used to extract antisense reads corresponding to the whole coding region. Reads were normalized to the library sizes. The mean normalized (mean of the three replicates) antisense reads and the reads upstream of the TSS and downstream of the TTS of the expressed, non-overlapping genes were plotted. Fig 2K shows the fitted degree 2-polynomial (ggplot2) of these data. TTSs generate significantly more antisense reads than TSSs in both convergent genes and others (non-convergent genes and intergenic regions) in Δ*xap5* mutant. (below, Welch's two sample t-test statistics for Δ*xap5*).

Comparision	Mean TSS	Mean TTS	p-value
All antisense	21.05	43.90	2.7e-05
All convergent	20.74	58.36	4.9e-04
All non-convergent	21.26	31.44	8.6e-03

Analysis of Faulty Transcription Termination in Δ*xap5*

To determine whether the antisense transcripts observed in the Δ*xap5* mutant could in part be due to read-through transcription/faulty termination at convergent gene pairs, we analyzed the data for sense transcription beyond 3'UTR into intergenic regions. We first extracted the sense reads for each gene 100 bp upstream of the transcription termination site (TTS). Since only genes with transcribed sense transcripts (upstream of TTS) could possibly have faulty termination issues, we normalized the upstream-TTS reads to the library size and filtered out genes with low expression (less than one read per one million counts in three or more samples). For those genes classified as expressed by this criterion, we extracted the sense reads mapping to the region 100 bp downstream of the TTS. After usual normalization and filtering, mean normalized reads were used to determine the genes with differential expression between the different genotypes immediately beyond the TTS.

ChIP-chip Analysis

ChIP-chip assays and data pre-processing were performed as previously described [15]. A *xap5*$^{+}$-HA-TAP construct in the endogenous *xap5* locus was introduced in the wild type background using the pFA6a-kanMX6-HATAP vector. This strain and the H2A.Z-FLAG tagged strain previously described in [15] were used for the ChIP-chip assays. Based on preliminary visual observations in IGB-6.2.2 [11] data were extracted for regions of interest for enrichment analyses. Enrichment analyses were performed as follows. In Fig. 4A and 4D, the H2A.Z/Pht1 and Xap5 protein association in repeated loci were compared relative to overall protein deposition of the protein of interest throughout the genome. In Fig. 4E, Xap5 protein association in loci with defective transcription

termination in the Δ*xap5* mutant was compared to that of all the genes in the genome. Here, the transcription start sites (TSS) and transcription termination sites (TTS) were defined as 300 base pairs upstream of TSS or downstream of TTS, respectively.

References

1. Hansen KR, Burns G, Mata J, Volpe TA, Martienssen RA, Bahler J, Thon G (2005) Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Mol Cell Biol* **25:** 590-601
2. Forsburg SL, Rhind N (2006) Basic methods for fission yeast. *Yeast* **23:** 173-183
3. Matsuyama A, Shirai A, Yashiroda Y, Kamata A, Horinouchi S, Yoshida M (2004) pDUAL, a multipurpose, multicopy vector capable of chromosomal integration in fission yeast. *Yeast* **21:** 1289-1305
4. Roguev A, Wiren M, Weissman JS, Krogan NJ (2007) High-throughput genetic interaction mapping in the fission yeast Schizosaccharomyces pombe. *Nat Methods* **4:** 861-866
5. Collins SR, Schuldiner M, Krogan NJ, Weissman JS (2006) A strategy for extracting and analyzing large-scale quantitative epistatic interaction data. *Genome Biol* **7:** R63
6. Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* **21:** 3448-3449
7. Sabatinos SA, Forsburg SL (2010) Molecular genetics of Schizosaccharomyces pombe. *Methods Enzymol* **470:** 759-795
8. Wang L, Si Y, Dedow LK, Shao Y, Liu P, Brutnell TP (2011) A low-cost library construction protocol and data analysis pipeline for Illumina-based strand-specific multiplex RNA-seq. *PLoS One* **6:** e26426
9. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25:** 1754-1760
10. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26:** 139-140
11. Nicol JW, Helt GA, Blanchard SG, Jr., Raja A, Loraine AE (2009) The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets. *Bioinformatics* **25:** 2730-2731
12. Quinlan AR, Hall IM (2010) BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26:** 841-842
13. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:** 402-408
14. Helmlinger D, Marguerat S, Villen J, Gygi SP, Bahler J, Winston F (2008) The S. pombe SAGA complex controls the switch from proliferation to sexual differentiation through the opposing roles of its subunits Gcn5 and Spt8. *Genes Dev* **22:** 3184-3195
15. Zofall M, Fischer T, Zhang K, Zhou M, Cui B, Veenstra TD, Grewal SI (2009) Histone H2A.Z cooperates with RNAi and heterochromatin factors to suppress antisense RNAs. *Nature* **461:** 419-422