

## **Yeast X-Chromosome Associated Protein 5 (Xap5) Functions with H2A.Z to Suppress Aberrant Transcripts**

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**Running Title:** Xap5 is a novel chromatin regulator

**Key words:** aberrant transcripts/chromatin modification/long terminal repeats/transcriptional repression/transposable elements

### **Abstract**

Chromatin regulatory proteins affect diverse developmental and environmental response pathways via their influence on nuclear processes such as the regulation of gene expression. Using a genome-wide genetic screen, we implicated a novel protein called X-chromosome associated protein 5 (Xap5) in chromatin regulation. We found that Xap5 is a chromatin-associated protein acting in a similar pathway to the histone variant H2A.Z to suppress expression of antisense and repeat element transcripts throughout the fission yeast genome. Xap5 is highly conserved across eukaryotes and a plant homolog rescues *xap5* mutant yeast. We conclude Xap5 likely functions as a chromatin regulator in diverse organisms.

## Introduction

Global regulation of transcription is key for organismal success, especially under stressful environmental conditions [1, 2]. Repression of aberrant transcripts such as transposons, long terminal repeats (LTRs), other repeat elements and antisense transcripts is as vital for success as the regulation of protein coding genes [3-5]. The accessibility of DNA to the transcriptional machinery is controlled by its association with histones and other chromatin proteins. Chromatin properties, and therefore transcription, are in large part determined by post-translational modifications of histones and the incorporation of variant histones in nucleosomes [6]. A genetic means of identifying previously unknown chromatin-associated genes is the epistatic miniarray profile (E-MAP) method [7, 8]. We recently carried out an E-MAP experiment assessing the genetic interactions of *X-chromosome associated protein 5 (xap5)* with approximately half of the genes encoded by the *Schizosaccharomyces pombe* genome.

Xap5-like proteins are highly conserved across eukaryotes [9]. These proteins are nuclear localized in yeast and plant cells [9, 10]. The human *XAP5* gene is located in the Xq28 region, where many disease genes map [11] and has trinucleotide CCG repeats in the 5' UTR, leading to the speculation that XAP5 may be implicated in human disease [12]. In addition, the paternally imprinted autosomal paralog of human *XAP5*, *XAP5-like* is located at 6p25.2 region and may play a role in spermatogenesis and carcinogenesis [13]. However the cellular function of Xap5 proteins has not been described in any organism. We now report that the *S. pombe* and *Arabidopsis thaliana* Xap5-like proteins are functionally conserved and Xap5 is a novel, evolutionarily conserved chromatin-binding protein that affects chromatin regulation in a manner similar to the variant histone H2A.Z.

## Results and Discussion

### Xap5 function is conserved between widely diverged eukaryotes

In order to characterize the function of a Xap5-domain protein in a simple eukaryote, we obtained *S. pombe* with a deletion in *xap5* (SPCC1020.12c). In minimal media (EMM50),  $\Delta xap5$  grows slightly more slowly than wild type at 30 °C and this is exacerbated under non-optimal growth temperatures (Fig 1A,B and Supplementary Fig S1 online). Xap5 family proteins are highly conserved across eukaryotes, suggesting that they may have similar molecular functions in diverse organisms [9]. We therefore tested whether the *Arabidopsis thaliana xap5* homolog *XCT* could rescue *S. pombe* mutant for *xap5*. The growth kinetics of wild type,  $\Delta xap5$  mutants transformed with either plant *XCT* ( $\Delta xap5$ -*XCT*), or *S. pombe xap5* ( $\Delta xap5$ -*xap5*) grown at 21 °C are virtually indistinguishable (Fig 1A,B). Indeed the generation time is almost two-fold longer for the  $\Delta xap5$  mutant than the *XCT* and *xap5* transformants (Fig 1A,B).

Given that *Arabidopsis* and fission yeast diverged more than one billion years ago [14] and that these two Xap5 homologs show the lowest levels of amino acid sequence conservation among the examined taxa with an identifiable Xap5 homolog [9], our findings suggest that Xap5 proteins are functionally well conserved across eukaryotes, sharing common molecular roles in diverse eukaryotes. A better understanding of the role

of Xap5 in fission yeast will therefore likely provide insight into the function of Xap5 homologs across eukaryotes.

### ***xap5* genetically interacts with known chromatin-associated genes**

We next performed a high-throughput genetic interaction analysis in *S. pombe* to obtain evidence regarding pathways in which *xap5* might function. Only a few mutants have significant genetic interactions with  $\Delta xap5$  at 30 °C, but many do when yeast are grown at suboptimal temperatures of 20 or 37 °C (Supplementary Dataset S1 online). Out of the 2117 tested, we found that 324 mutants have significant genetic interactions with *xap5* at 37 °C and 172 at 20 °C. Importantly, there is considerable overlap between the significantly interacting mutants at these two temperatures (Fisher's exact test,  $p < 2.2e-16$ ) and the gene ontology (GO) distributions for biological process and the genetic interaction score (S-score) profiles are also similar (Supplementary Fig S2 online). To identify pathways in which Xap5 might act, we looked for enrichment of particular GO terms among mutants that significantly interact with  $\Delta xap5$  or that have similar genetic interaction profiles. Mutants that positively interact with  $\Delta xap5$  at 37 °C are enriched for histone modification (GO ID 16570,  $p=0.035$ ) and covalent chromatin modification (GO ID 16569,  $p=0.035$ ) functions.

In addition, of the 953 mutants (872 loci) previously subjected to E-MAP analysis, those with similar genetic interactions as  $\Delta xap5$  (positively correlated genes; Supplementary Dataset S1 online) are enriched for roles in chromatin modification-mediated transcriptional control (Supplementary Table 1, Fig 1C). Individual mutants with genetic interaction profiles similar to  $\Delta xap5$  include genes that function in well-known chromatin remodeling protein complexes including multiple members of both the Set1C/COMPASS complex, which is involved in H3K4 methylation, and the Swr1C complex, which catalyzes the deposition of the histone variant H2A.Z (encoded by the *pht1* gene) (Fig 1C–E, Supplementary Table S2 online). Genes that act in similar pathways often have positively correlated genetic interaction profiles [15], suggesting Xap5 is involved in chromatin regulation or maintenance. Since multiple Pht1/H2A.Z mutants are significantly correlated with  $\Delta xap5$  we compared genome-wide gene expression profiles of yeast with deletions in either *xap5* or *pht1*, as described in more detail below. The  $\Delta pht1$  and  $\Delta xap5$  mutants have well-correlated gene expression profiles (Fig 1F), indicating that Xap5 and Pht1/H2A.Z regulate similar loci. Together, the genetic and gene expression data strongly suggest that Xap5 plays a role in chromatin modification.

We wished to confirm the genetic interactions between  $\Delta xap5$  and selected chromatin mutants with positively correlated genetic interactions (Supplementary Table S2 online). Like  $\Delta xap5$  mutants,  $\Delta pht1$  mutants have a subtle slow growth phenotype in EMM50 at 30 °C that is aggravated at 37 °C and 20 °C and exacerbated when this mutant is combined with the  $\Delta xap5$  mutation (Fig 1G,H and Supplementary Fig S3 online). Like *pht1*, *set1* (which encodes an H3K4 methyltransferase) is also statistically significantly genetically correlated with *xap5* (Fig 1C,E). However, there is an antagonistic genetic interaction between the *set1* and *xap5* mutants (Supplementary Fig S3E online). Together,

these data suggest that Xap5 plays a role in chromatin modification, acting in a similar pathway to Pht1/H2A.Z and antagonistically with Set1.

### **Antisense, intergenic and other non-coding transcripts are misexpressed in the absence of Xap5**

Since both Set1 and Pht1/H2A.Z play roles in the regulation of transcription, we carried out RNA-seq analysis to characterize the transcriptome of *Δxap5* mutants. As previously reported for *Δpht1* [16], we found that hundreds of loci corresponding to multiple types of transcripts are misregulated in both *Δxap5* and *Δpht1* (Supplementary Dataset S2 online; Fig 2A) and that the transcriptomes of *Δxap5* and *Δpht1* mutants are positively correlated (Fig 1F). The Arabidopsis *XCT* gene almost completely rescues the misregulation of gene expression in *Δxap5* (Fig 2A,B), consistent with its ability to rescue the *Δxap5* growth phenotype (Fig 1A,B). The *Δxap5Δpht1* double mutant displays a synergistic increase in all types of misregulated transcripts relative to either single mutant (Fig 2A,B), consistent with Xap5 and Pht1/H2A.Z playing similar but not identical roles. The misregulated loci include protein coding (both sense and antisense), intergenic, and other non-coding sequences. Notably, a higher fraction of the misexpressed loci in both *Δxap5* and *Δpht1* correspond to antisense, intergenic, or other non-coding transcripts than to sense protein coding transcripts (Fig 2A,B).

More than 80% of the loci significantly misregulated ( $p \leq 0.01$ , fold change  $\geq 2$ ) in *Δxap5*, including both coding and non-coding transcripts, are upregulated (Fig 2C–H and Supplementary Fig S4A–H online). Upregulated genes are significantly enriched for GO categories involved in stress responses (Supplementary Table S3 online) whereas downregulated genes are enriched for diverse biological processes including translation and processing of non-coding RNAs (Supplementary Table S4 online). The temperature-dependent slow-growth phenotypes and upregulation of stress-related transcripts in *xap5* mutants indicate that Xap5 function is especially important in adverse environmental conditions. As expected given the positive correlation between the transcriptomes of the *Δxap5* and *Δpht1* mutants (Fig 1F), many loci upregulated in *Δxap5* are also upregulated in *Δpht1* and *Δxap5Δpht1* (Fig 2C–H and Supplementary Fig S4A–H online). Centromeres, telomeres and silent mating-type loci are not expressed in either the *Δxap5* or the *Δpht1* mutant, indicating that Xap5 and Pht1/H2A.Z are not essential for maintenance of strongly heterochromatic regions. Consistent with this, neither Xap5 nor Pht1/H2A.Z [16] association with regions of constitutive heterochromatin is enriched relative to the rest of the genome (Supplemental Data S3 online).

Antisense transcripts are prominently upregulated in *Δxap5* and *Δpht1* and are synergistically overexpressed in *Δxap5Δpht1* (Fig 2A,B). There are different patterns of correlation between the sense and the antisense transcripts of individual genes suggesting that the misregulation of sense transcripts in *Δxap5* is not primarily due to misregulation of antisense transcripts (Supplementary Fig S4 online). Many (54%) of the antisense transcripts in *Δxap5* and more than 90% of the antisense transcripts in *Δpht1* [16] map to convergent loci, suggesting these may have resulted from inappropriate read-through transcription (Fig 2I). We confirmed that this is the case for one locus using strand-

specific RT-PCR (Fig 2J). Consistent with a general role for read-through transcription in the generation of antisense transcripts, we observed more upregulation of antisense transcripts near transcription termination sites than near transcription start sites (Fig 2K, Supplementary Information Online). Of the 4240 detectably expressed genes, 178 and 113 display faulty transcriptional termination in  $\Delta xap5$  and  $\Delta pht1$  mutants, respectively (Supplementary Dataset S2 online; see Online Methods for analysis details). 38 of these inappropriately terminated transcripts are shared between the two single mutants (significantly more overlap than expected by chance;  $p = 6.7 \text{ e-}15$ , Fisher's exact test). This suggests that Xap5 is somehow required for proper transcription termination. Further strengthening this possibility, as described in more detail below we found that Xap5 protein is preferentially associated with the gene bodies and transcription termination sites of loci with faulty transcription termination in  $\Delta xap5$  mutants (Fig 4E).

Spurious antisense reads can be generated during library synthesis due to second-strand cDNA synthesis during reverse transcription, an event that can be inhibited by the addition of actinomycin D [17], albeit at the expense of decreased 5' and 3' coverage of transcripts and increased variation in overall expression levels [18]. Although we did not add actinomycin D during library synthesis, the reproducible detection of increased antisense levels in the mutants relative to wild type (Fig. 2B), the inverse correlations between changes in expression for many sense and antisense transcripts (Fig. S4I, J), and our verification of upregulation of many antisense transcripts using locus-specific primers (Figs. 2J, S4G, S5C) support our conclusion that Xap5 and Pht1/H2A.Z cooperate to suppress expression of a variety of aberrant sequences including antisense and read-through transcripts at loci throughout the *S. pombe* genome. Indeed, the  $\Delta xap5 \Delta pht1$  double mutants display an enhanced increase in antisense transcripts (Fig 2B) very similar to that seen when the  $\Delta pht1$  mutant is combined with mutations in several different silencing complexes [16].

### **Xap5 and H2A.Z/Pht1 binds chromatin in repeat element loci to repress their transcription**

Along with upregulation of protein coding transcripts, many repeat elements, including retrotransposons (*Tf2* elements), LTRs, and *wtf* elements are upregulated in  $\Delta xap5$ ,  $\Delta pht1$  and  $\Delta xap5 \Delta pht1$  (Fig 3). Out of the 238 LTRs annotated in the *S. pombe* genome, 117 are detectably expressed in wild type and all three mutants (Supplementary Dataset S2 online). Of these 117 LTRs, 21 (~18%) and 28 (24%), are significantly ( $p \leq 0.05$ ) upregulated in  $\Delta xap5$  and  $\Delta pht1$ , respectively. This number increases synergistically to 79 (~67.5%) in the double mutant (Fig 3A,B). Of 25 *wtf*s in the *S. pombe* genome, 17 and 22 are significantly ( $p \leq 0.01$ ) upregulated in the  $\Delta xap5$  and  $\Delta xap5 \Delta pht1$  mutants respectively (Fig 3C,D and Supplementary Fig S4H online). Relative to all genes, these different types of repeat loci are preferentially upregulated in both  $\Delta xap5$  and  $\Delta pht1$  mutants (Fisher's exact test, p-values for LTRs =  $1.203\text{e-}05$  and *wtf*s  $< 2.2\text{e-}16$  in  $\Delta xap5$  and LTRs  $< 2.2\text{e-}16$  and *wtf*s =  $0.006443$  in  $\Delta pht1$ ). This pattern suggests that Xap5 and Pht1/H2A.Z are involved in silencing these repeat elements.

Since Xap5 affects processes regulated by known chromatin-associated proteins, we investigated whether Xap5 itself binds chromatin using chromatin immunoprecipitation followed by microarray analysis (ChIP-chip). We found that Xap5 protein is associated with chromatin in both genic and intergenic regions (Supplementary Dataset S3 online). Both Xap5 and Pht1/H2A.Z are significantly enriched at all 13 *Tf2* transposable element loci (Fig 4A,B). Consistent with these associations having functional significance, *Tf2* ORFs are upregulated in both  $\Delta xap5$  and  $\Delta pht1$  mutants (Fig 3E, Fig 4B and Supplementary Table S5). Many LTRs and *wtf*s are significantly enriched for association of both Xap5 and H2A.Z (Fig 4A,C) and these and adjacent loci have altered expression in both mutants (Fig 4C, Supplementary Table S5 and Fig 3A–D).

We next investigated whether Xap5 and Pht1/H2A.Z association with chromatin is interdependent. We found no significant alteration of Pht1/H2A.Z binding to chromatin in  $\Delta xap5$  mutants and no global change in Xap5 association with chromatin in  $\Delta pht1$  mutants (Fig 4D and Supplementary Dataset S3 online). However, we found an appreciable increase in Xap5 association with *Tf2* and *wtf* loci in  $\Delta pht1$  mutants relative to wild type (Fig 4D, Supplementary Dataset S3 online). These observations suggest Xap5 and Pht1 cooperatively contribute to the repression of transcription of repeat elements such as *wtf*s and *Tf2*s, such that loss of both genes causes synergistic upregulation of expression of these loci.

Similar upregulation of cryptic transcripts including repeat elements has previously been observed in silencing and RNAi pathway mutants in fission yeast [4, 19, 20] and chromatin regulatory gene mutants in budding yeast [21-23]. Moreover, the combination of such mutants with  $\Delta pht1$  has previously been shown to synergistically increase expression of antisense RNAs in fission yeast [16]. We therefore compared the  $\Delta xap5$  transcriptome with previously published microarray data for several gene silencing mutants (*clr6.1*, *clr4.681* and *clr6.1 $\Delta$ clr3*) and one RNAi mutant ( $\Delta dcr1$ ) [4]. We found moderate but statistically significant levels of correlation between the transcriptomes of  $\Delta xap5$  and all these mutants, with the highest correlation seen between  $\Delta xap5$  and *clr6.1 $\Delta$ clr3*, which have mutations in genes encoding histone deacetylases (Supplementary Fig S5A,B online). These correlations might well be higher if not for the differences in growth conditions, gene expression analysis platforms, and library construction methods between the experiments being compared [4]. Since many types of non-coding transcript loci are upregulated in different histone deacetylase complex mutants [24] we examined the expression of several antisense and intergenic loci that are misexpressed in  $\Delta xap5$  and  $\Delta pht1$  in five different histone deacetylase complex mutants including  $\Delta clr3$  and *clr6-1*. Strand specific RT-PCR revealed that these loci are upregulated in all of these mutants as well as in  $\Delta xap5$  and  $\Delta pht1$  (Supplementary Fig S5C online).

Though the H3K9 methyltransferase Clr4, the histone deacetylases Clr6 and Clr3, and the RITS component Dcr1 are best known for their roles in silencing of heterochromatic regions, these proteins are also involved in the regulation of euchromatic loci [4, 19, 25]. Many genes involved in environmental stress responses are derepressed in the *clr* mutants [4], similar to what we observe in  $\Delta xap5$ . In addition, the above Clr proteins are required

for the silencing of retrotransposons, LTRs, and *wtf*s [4, 19]; notably, these types of loci are also enriched for Xap5 association and are derepressed in  $\Delta xap5$ . Moreover Clr6 is a component of the Rpd3L complex [26], other members of which have significantly correlated genetic interactions with *xap5* (Supplementary Table S2 online). Therefore, overall these correlations further support our conclusion that Xap5 cooperates with well-studied protein complexes to regulate chromatin.

## Conclusions

Xap5 proteins lack recognizable functional domains, making prediction of their molecular functions difficult [9, 12]. However, our genome-wide investigation has allowed us to assign a deeply conserved role for Xap5 in the regulation or maintenance of euchromatin. Notably, hundreds of loci are misregulated in the  $\Delta xap5$  mutant (Fig 2), indicating that it is indeed required for appropriate transcriptional regulation across the *S. pombe* genome. However, Xap5 is not likely to encode a typical transcription factor: most misexpressed sequences in  $\Delta xap5$  are upregulated, and among these 60% represent aberrant transcripts such as antisense and intergenic sequences while only 36% correspond to protein coding gene transcripts (including the *wtf*s, a family of genes of unknown function). Therefore the primary function of Xap5 appears to be the genome-wide transcriptional silencing of antisense and intergenic and repeat element loci. Several distinct classes of factors have previously reported to be involved in such repression, including H2A.Z, the RNAi machinery, chromatin remodelers, histone methyltransferases, and the exosome [16, 21, 22, 25, 27-29]. A precise determination of the molecular function of the conserved chromatin regulatory Xap5 proteins therefore awaits further, likely biochemical, studies.

## Methods

Additional details are included in Supplementary Information Online.

### Growth Analysis and Gene Transformations in *S. pombe*

Strains used are listed in Supplementary Information. All yeast media and standard methods were according to Forsburg and Rhind [30].

### Genetic Interaction Analysis

The epistatic miniarray profile (E-MAP) method was used [8, 31]. Cells were grown at 20, 30 or 37 °C in YES media.

### RNA-seq, RT-PCR and ChIP-chip Analyses

Details and all primers used are listed in Supplementary Information Online.

### Data Access

All raw data files are available at the NCBI Gene Expression Omnibus (GEO) repository under the accession number GSE46506.

### Acknowledgements

We thank Dr. Nicholas R. Rhind (University of Massachusetts) for initial assistance and advice; Dr. Kaz Shiozaki and Dr. Hisashi Tatebe (NAIST, Japan) for the wild-type strain



and expert advice; and Dr. Julin Maloof (University of California Davis) for assistance with RNA-seq data analysis. This work was supported by National Institutes of Health grant R01 GM069418 to SLH.

### **Author contributions**

S.A. and S.L.H. designed research; S.A., A.R., and M.Z. performed research; S.A., A.R., M.Z., N.J.K., and S.I.S.G. contributed new reagents/analytic tools; S.A., A.R., M.Z. and S.L.H. analyzed data; and S.A. and S.L.H. wrote the paper.

### **Conflict of Interest**

The authors declare that they have no conflict of interest.

Supplementary information is available at EMBO reports Online.

### **References**

1. Weiner A *et al* (2012) Systematic dissection of roles for chromatin regulators in a yeast stress response. *PLoS Biol* **10**: e1001369
2. Zhu Y, Dong A, Shen WH (2012) Histone variants and chromatin assembly in plant abiotic stress responses. *Biochim Biophys Acta* **1819**: 343-348
3. Lorenz DR, Mikheyeva IV, Johansen P, Meyer L, Berg A, Grewal SI, Cam HP (2012) CENP-B cooperates with Set1 in bidirectional transcriptional silencing and genome organization of retrotransposons. *Mol Cell Biol*, 10.1128/MCB.00395-12
4. 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
5. Cam HP, Sugiyama T, Chen ES, Chen X, FitzGerald PC, Grewal SI (2005) Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. *Nat Genet* **37**: 809-819
6. Berger SL (2007) The complex language of chromatin regulation during transcription. *Nature* **447**: 407-412
7. Roguev A *et al* (2008) Conservation and rewiring of functional modules revealed by an epistasis map in fission yeast. *Science* **322**: 405-410
8. 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
9. Martin-Tryon EL, Harmer SL (2008) XAP5 CIRCADIAN TIMEKEEPER coordinates light signals for proper timing of photomorphogenesis and the circadian clock in *Arabidopsis*. *Plant Cell* **20**: 1244-1259
10. Matsuyama A *et al* (2006) ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol* **24**: 841-847
11. Kolb-Kokocinski A, Mehrle A, Bechtel S, Simpson JC, Kioschis P, Wiemann S, Wellenreuther R, Poustka A (2006) The systematic functional characterisation of Xq28 genes prioritises candidate disease genes. *BMC Genomics* **7**: 29

12. Mazzarella R, Pengue G, Yoon J, Jones J, Schlessinger D (1997) Differential expression of XAP5, a candidate disease gene. *Genomics* **45**: 216-219
13. Zhang A, Skaar DA, Li Y, Huang D, Price TM, Murphy SK, Jirtle RL (2011) Novel retrotransposed imprinted locus identified at human 6p25. *Nucleic Acids Res* **39**: 5388-5400
14. Sipiczki M (2000) Where does fission yeast sit on the tree of life? *Genome Biol* **1**: REVIEWS1011
15. Collins SR *et al* (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* **446** 5
16. 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
17. Perocchi F, Xu Z, Clauder-Munster S, Steinmetz LM (2007) Antisense artifacts in transcriptome microarray experiments are resolved by actinomycin D. *Nucleic Acids Res* **35**: e128
18. Levin JZ, Yassour M, Adiconis X, Nusbaum C, Thompson DA, Friedman N, Gnirke A, Regev A (2010) Comprehensive comparative analysis of strand-specific RNA sequencing methods. *Nat Methods* **7**: 709-715
19. Woolcock KJ, Gaidatzis D, Punga T, Buhler M (2011) Dicer associates with chromatin to repress genome activity in *Schizosaccharomyces pombe*. *Nat Struct Mol Biol* **18**: 94-99
20. Hennig BP, Bendrin K, Zhou Y, Fischer T (2012) Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription. *EMBO Rep* **13**: 997-1003
21. Cheung V, Chua G, Batada NN, Landry CR, Michnick SW, Hughes TR, Winston F (2008) Chromatin- and transcription-related factors repress transcription from within coding regions throughout the *Saccharomyces cerevisiae* genome. *PLoS Biol* **6**: e277
22. van Bakel H, Tsui K, Gebbia M, Mnaimneh S, Hughes TR, Nislow C (2013) A compendium of nucleosome and transcript profiles reveals determinants of chromatin architecture and transcription. *PLoS Genet* **9**: e1003479
23. Whitehouse I, Rando OJ, Delrow J, Tsukiyama T (2007) Chromatin remodelling at promoters suppresses antisense transcription. *Nature* **450**: 1031-1035
24. Nicolas E, Yamada T, Cam HP, Fitzgerald PC, Kobayashi R, Grewal SI (2007) Distinct roles of HDAC complexes in promoter silencing, antisense suppression and DNA damage protection. *Nat Struct Mol Biol* **14**: 372-380
25. Zhang K, Fischer T, Porter RL, Dhakshnamoorthy J, Zofall M, Zhou M, Veenstra T, Grewal SI (2011) Clr4/Suv39 and RNA quality control factors cooperate to trigger RNAi and suppress antisense RNA. *Science* **331**: 1624-1627
26. Shevchenko A *et al* (2008) Chromatin Central: towards the comparative proteome by accurate mapping of the yeast proteomic environment. *Genome Biol* **9**: R167
27. Buhler M, Spies N, Bartel DP, Moazed D (2008) TRAMP-mediated RNA surveillance prevents spurious entry of RNAs into the *Schizosaccharomyces pombe* siRNA pathway. *Nat Struct Mol Biol* **15**: 1015-1023
28. DeGennaro CM, Alver BH, Marguerat S, Stepanova E, Davis CP, Bahler J, Park PJ, Winston F (2013) Spt6 regulates intragenic and antisense transcription, nucleosome

positioning, and histone modifications genome-wide in fission yeast. *Mol Cell Biol* **33**: 4779-4792

29. Shim YS, Choi Y, Kang K, Cho K, Oh S, Lee J, Grewal SI, Lee D (2012) Hrp3 controls nucleosome positioning to suppress non-coding transcription in eu- and heterochromatin. *EMBO J* **31**: 4375-4387

30. Forsburg SL, Rhind N (2006) Basic methods for fission yeast. *Yeast* **23**: 173-183

31. 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

32. Kim HS *et al* (2009) An acetylated form of histone H2A.Z regulates chromosome architecture in *Schizosaccharomyces pombe*. *Nat Struct Mol Biol* **16**: 1286-1293

## Figure Legends

**Fig 1:  $\Delta xap5$  genetic interaction profile is correlated with that of known chromatin modifiers.** (A, B) Yeast  $\Delta xap5$  mutants grown in EMM50 at 21 °C exhibit a slow growth phenotype rescued by the Arabidopsis *XCT* gene. Mean $\pm$ s.e.m. of at least three independent experiments with at least three replicates ( $n\geq 3$ ) (C) Frequency distribution plot of correlation coefficients of the *xap5* mutant genetic interaction profile with that of other mutants. Red=members of Set1C/COMPASS and black=members of Swr1C. In the two *pht1* mutant alleles shown, the four lysines (4K) at the N-terminus were mutated to unacetyltable residues, disrupting their function [32]. (D) *pht1* and (E) *set1* mutant genetic interaction (S-score) profiles vs. that of the *xap5* mutant (Pearson,  $p < 2.2e-16$ ). (F) Transcriptome profiles of  $\Delta xap5$  vs.  $\Delta pht1$  (Spearman,  $r=0.53$ ,  $p < 2.2e-16$ ). *H2A.Z/pht1* genetically interacts with *xap5*. (G, H) Growth curves for  $\Delta xap5$ ,  $\Delta pht1$  and  $\Delta xap5\Delta pht1$  mutants in EMM50 at 37 °C. Mean $\pm$ s.e.m. of at least three independent experiments with at least three replicates ( $n\geq 3$ ). B and H, one-way ANOVA with Bonferroni's multiple comparison test, \* indicates  $p=0.05$ , \*\* indicates  $p=0.0001$ .

**Fig 2: Aberrant transcripts are upregulated in  $\Delta xap5$ .** (A) Types of transcripts differentially expressed in the indicated genotypes. (B) Percent total antisense reads (antisense reads as a fraction of total reads per library) in the mutants. One-way ANOVA with Bonferroni's multiple comparison test, \* indicates  $p=0.05$ , \*\* indicates  $p=0.03$ , mean $\pm$ s.e.m of two lanes with three biological replicates ( $n=3$ ). Percentage of protein coding genes (C) sense and (D) antisense transcripts up- or down-regulated in  $\Delta xap5$ . Venn diagrams showing the overlap between (E) upregulated and (F) downregulated sense protein coding genes and (G) upregulated and (H) downregulated antisense transcripts in the indicated mutants. (I) Read-through transcription at the *rps2402-cnt5/csx2* locus generates antisense transcripts identified by RNA-seq. (J) Strand-specific RT-PCR confirms the presence of a read through transcript (RC3'). Similar results were observed at least in two independent experiments. (K) Genome-wide abundance of antisense transcripts is significantly higher in all three mutants at transcription termination sites (TTS) than transcription start sites (TSS) (Welch's two sample t-test,  $p=0.002$ ). The values are the means of three biological replicates ( $n=3$ ). Statistical significance of differential expression was determined using the negative binomial exact test ( $p\leq 0.01$ , fold change $\geq 2$ ).

**Fig 3: Many repeat elements are significantly upregulated in the  $\Delta xap5$  mutant** (A) LTRs significantly upregulated ( $p\leq 0.05$ ) in the  $\Delta xap5$  mutant and (B) overlap between upregulated LTRs among different genotypes. (C) *wtf* elements significantly upregulated ( $p\leq 0.01$ ) in the  $\Delta xap5$  mutant and (D) the overlap between upregulated *wtf*s among different genotypes. A and C, mean $\pm$ s.e.m. of three biological replicates ( $n=3$ ) with data combined from two independent lanes (E) *Tf2* transposable element ORFs are upregulated in the mutants (quantitative RT-PCR, letters denote statistically significantly different groups, one-way ANOVA with Bonferroni's multiple comparison test,  $p<0.01$ ,

mean±s.e.m. of three biological replicates (n=3); experiment was repeated twice with similar results).

**Fig 4: Xap5 and Pht1/H2A.Z protein association with chromatin is enriched at repeat element loci.** (A) Association of both Pht1/H2A.Z and Xap5 proteins are significantly enriched at LTR, transposable element (*Tf2s*) and *wtf* loci compared to the overall genome deposition levels (H2A.Z.all and Xap5.all). (B) Transposable element and (C) *wtf* loci are enriched for association of both Pht1/H2AZ and Xap5 proteins. Sense transcription from reverse and forward strands is shown in B and C, respectively. Sense transcripts are upregulated in both mutants at these and adjacent loci (see also Supplementary Table S5). (D) Association of Xap5 protein is increased at *Tf2* and *wtf* loci in  $\Delta$ *pht1* relative to wild type. (E) Association of Xap5 protein is significantly enriched in gene bodies and transcription termination sites (TTS) but not transcription start sites (TSS) of protein coding loci with defective transcription termination in the  $\Delta$ *xap5* mutant relative to all the genes in the genome. A, D and E - Welch's two sample t-test (\* indicates  $p=0.004$ , \*\* indicates  $p=2.2e-4$ , and \*\*\* indicates  $p\leq 1.2e-15$ ). Data are from one experiment; the same trend was observed in two independent biological replicates.









