Supplementary data S1

S. pombe strains used in this study

Strain	Genotype	Source
Hu0303	*h⁻ wild type*	
Hu0029	*h⁻ ade6-M210 ura4-D18 leu1-32*	R. Allshire
FY0412	*h⁺ cc2 (SphI)::ura4⁺ ade6-M210 leu1-32 ura4-DS/E*	R. Allshire
FY0498	*h⁺ imr1*R *(Nco*I*)::ura4⁺ orientation I ade6-M210 leu1-32 ura4-DS/E*	R. Allshire
FY0597	*h⁹⁰ mat3-M(Eco*RV*)::ura4⁺ ade6-M210 leu1-32 ura4-DS/E*	R. Allshire
FY0965	*h⁺ otr1*R *(dhNdeI-BglII)HindIII::ura4⁺ ade6-M210 leu1-32 ura4-DS/E*	R. Allshire
FY1862	*h⁺ ade6-M210 his3-D1 leu1-32 ura4-D18 otr1RSphI::ade6⁺ his3-tel(1L) ura4⁺::TEL (2L)*	R. Allshire
Hu0393	*h⁺ leu1/Ylp2.4pUCura4⁺.7 ade6-M216 leu1-32 ura4-DS/E*	K. Ekwall
Hu1103	*h⁻ hst4Δ::his3⁺ his3-D1*	This study
Hu1251	*h⁻ hst2Δ::kanMx4*	This study
Hu1098	*h⁻ sir2Δ::kanMx6*	K. Ekwall
Hu1481	*h⁻ hst4Δ::kanMx4*	This study
LPY04586	h⁻ *sir2-myc::kanMX6 ade6-M210 arg3-D4 his3-D1 leu1-32 ura4-D18*	L. Pillus
Hu1503	*h⁻ hst2-myc::kanMX4 ade6-M210 leu1-32 ura4-D18*	This study
LPY4012	*h⁻ ade6-M216 arg3-D4 his3-D1 leu1-32 ura4-D18 hst4Δ::his3⁺ + pLP1093 Leu2⁺ protA-hst4*	L. Pillus
Hu1627	*h⁾ hst2-myc::kanMX6 POM152-GFP::KanMx6 ade6-M210 leu1-32 ura4-D18*	This study
Hu1409	*h⁺ hst2Δ::kanMX6 Ylp2.4pUCura4.7 ade6-M216 leu1-32 ura4-DS/E*	This study
Hu1430	*h⁺ hst4Δ::kanMX6 Ylp2.4pUCura4.7 ade6-M216 leu1-32 ura4-DS/E*	This study
Hu1408	*h⁺ sir2Δ::kanMX4 Ylp2.4pUCura4.7 ade6-M216 leu1-32 ura4-DS/E*	This study
Hu1585	*h⁺ hst2Δ::kanMX4 otr1 (dh NdeI- BglII)Hin*dIII*::ura4⁺ ade6-M210 leu1-32 ura4-D18*	This study
Hu1523	*h⁺ hst2Δ::kanMX4 ade6-M210 leu1-32 ura4-D18 Ura4⁺::TEL1*	This study
Hu1589	*h⁺ hst2Δ::kanMX4 cc2 (SphI)::ura4⁺ ade6-M210 leu1-32 ura4-D18*	This study
Hu1587	*h⁺ hst2Δ::kanMX4 imr1R (NcoI)::ura4⁺ orientation I ade6-M210 leu1-32 ura4-D18*	This study
Hu1405	*h⁹⁰ hst2Δ::kanMX4 mat3-M(EcoRV)::ura4⁺ ade6-M210 leu1-32 ura4-DS/E*	This study
Hu1401	*h⁻ hst2Δ::kanMX4 sir2Δ::kanMX4*	This study
Hu1474	*h⁻ hst2Δ::kanMX4 hst4Δ::kanMX4 ade6-M216 leu1-32 ura4-D18*	This study
Hu1476	*h⁻ hst4Δ::kanMX4 sir2Δ::kanMX4 ade6-M216 leu1-32 ura4-D18*	This study

Microarrays

All the microarray data in this study have been submitted to Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo/ with accession number GSE6114. The combined microarray strategy in this study was performed essentially as outlined in (Wiren et al., 2005). cDNA expression profiling of *hst2*Δ mutant was carried out according to (Xue et al., 2004). We used the *S. pombe* ORF and combined IGR+ORF (4976 ORF and about 5000 IGR probe fragments including 4960 promoter IGR fragments, representing all RNA pol II promoter regions, 20 tRNA gene promoter fragments, 4 fragments for different rDNA promoters (5S,

18S, 5.8S and 28S), and 11 fragments for non-coding centromere repeats *dg1, dh, imr, cnt1, cnt2, cnt3*) spotted microarrays (Eurogentec, Belgium custom DNA microarray services). For histone acetylation maps, ChIP-CHIP method was essentially used using according to (Robyr and Grunstein, 2003). Antibodies against H3K9Ac, H3K14Ac, H4K5Ac, H4K16Ac, H4K12Ac (Suka et al., 2002) were used. The histone 'H3cter' antibody was purchased from Upstate and used for ChIP according to manufacturers recommendations. For the Hst2-myc binding map experiments we used the ChIP-CHIP procedure described by (Kurdistani et al., 2002). For ProtA-Hst4 binding map experiments immunoprecipitation was performed with rabbit IgG sepharose beads (Sigma). Corrections for nucleosome loss were performed as described in (Wiren et al., 2005). GeneSpring software was used for all data analysis. Expression profiling and histone acetylation mutant *versus* wt data sets were normalized using Lowess (per spot per chip) intensity-dependent normalization, which corrects nonlinear rates for dye incorporation. Cut off values of 1.5 were used to generate 'high' and 'low' gene lists for ORF and IGR regions. The Hst2-myc and protA-Hst4 binding data sets were subjected to 'per chip' normalization using the 50^{th} percentile.

Similar gene lists were identified using the automatic hyper-geometric distribution tests in the Gene List inspector function of Gene Spring. The hyper-geometric distribution test calculates the probability of overlap corresponding to k or more IGR or ORF fragments between an IGR or ORF list of n fragments compared against another gene list of m fragments when randomly sampled from a universe of u genes:

$$\frac{1}{\binom{u}{m}} \sum_{i=k}^{n} \binom{m}{i} \binom{u-m}{n-i}$$

The significantly overlapping gene lists were illustrated using Venn diagrams. Different gene ontology categories of microarrays data were determined using the GoMiner web resource (http://discover.nci.nih.gov/gominer/). In GoMiner two sided Fisher's exact tests are used to test statistical significant enrichment in a perticular gene ontology category (Zeeberg et al., 2003).

Immunofluorescence microscopy

S. pombe cells were prepared for immunofluorescence microscopy according (Bjerling *et al.* 2002) with primary antibodies mouse anti-myc (Sigma) and rabbit anti-GFP (Jackson Immunoresearch Laboratories). Fluorescein isothiocyanate- or Texas red- conjugated

secondary antibodies were purchased from Jackson Immunoresearch Laboratories. Cells were visualised with a Zeiss Axioplan II microscope equipped with a Hamamatsu C4742-95 charge-coupled device camera. A z-series digital confocal deconvolution analysis with Openlab software, version 4.0.2 (Improvision) was performed with 0.3 µm sample z-series spacing and nearest-neighbor deconvolution. An object magnification of x 100 and a lens aperture of l .4 were used.

RNA analysis

For northern blotting, total RNA of Hu0303 (wt), Hu1103 (*hst4*Δ), Hu1098 (*sir2*Δ) and Hu1251 (*hst2*Δ) was extracted according (Xue et al., 2004), and treated with Shrimp DNAase I (Amersham), 10µg total RNA (DNA-free) were separated on formaldehyde- agarose gels (0.8%) and transferred by capillarity blotting to N+-Hybond nylon membranes (Amersham). ^{32}P-dCTP DNA probes were prepared by random priming using the Megaprime DNA labelling System kit (Amersham). Tf2 template DNA was amplified by PCR using the following primers: forward primer (+ 47 to +66) 5'-GGAGACCAGAGAATTTGGAT-3' and reverse primer (+1107 to +1125) 5'-TGGTAACTCAGGTTCCTTG-3'. The PCR product was gel- purified and used as a template for probe preparation. After UV cross-linking, the membranes were blocked in 'PreHyb' buffer (Amersham) for 30 min at 65 ^{o}C and hybridized with Tf2 radioactive probes overnight at 65°C. The membranes were washed once for 5 min in 2x SSC, 15 min in 2xSSC 0.l % SDS at 50 ^{o}C, 15 min in 0.2xSSC 0.1% SDS at 50 ^{o}C and 5 min with 2xSSC at room temperature. The membranes were then scanned by FLA-3000 analysis (Fujifilm) and visualised using Image Gauge V.4.0. software (Fujifilm).
Quantitative RT-PCR was performed using 50 ng of total RNA (DNA-free) extracted from Hu0303, Hu1103 and Hu1098 strains as described above. RNA samples were amplified using iSCript^{TM} One-step RT-PCR kit with SYBR green (Bio-Rad) using a 'ICycler' thermocycler (Bio-Rad) and visualised with 'MyIQ' Software (Bio-Rad) according the recommendation of the manufacturer. RT-PCR amplification were performed with the following primers (+ and - indicate the position relative to the start codon): Actin l (SPBC32H8 .12c) forward primer (+445 to + 464) 5'-ACTGGTATCGTCTTGGACTC-3' and reverse primer (+994 to + 975) 5'-GAGCAACAATCTTGACCTTC-3'; Glutamyl-tRNA synthetase (SPAPB1A10.11c) forward primer (+413 to +431) 5'-ATTCGGTCCCTATCGCTAC-3' and reverse primer (+732 to +711) 5'-CCATTCCTCTCCTCTAATAACG-3'; Tf2 fragment l forward primer (-155 to -134) 5'-CTTGTGATCTACAATTAACTCC-3' and reverse primer (+47 to +66) 5'-ATCCAAATTCTCTGGTCTCC-3'; Tf2 fragment 2 forward primer (-53 to -73) 5'-

CTGGGTTCAAAGGAGAAGGAA-3' and reverse primer (+47 to +66) 5'-ATCCAAATTCTCTGGTCTCC-3'; Tf2 fragment 3 forward primer (+767 to +786) 5'-AAGCTGAACTTCCAGACTTC-3' and reverse primer (+1125 to +1107) 5'-TGGTAACTCAGGTTCCTTG-3'.

5' Rapid Amplification of cDNA Ends (RACE)

5'-RACE reactions were performed using 2µg of total RNA extracted of Hu1103 (*hst4*Δ) and Hu0303 (wt) strains using a 5'-RACE (rapid amplification of cDNA ends) kit according the manufacturer's recommendations (Invitrogen). After a reverse transcription step with Tf2-specific primer GSP1 (- 1125 to - 1107) 5'-TGGTAACTCAGGTTCCTTG-3' and reverse transcriptase, the cDNA was tailed with terminal deoxynucleotidyl transferase and was subsequently amplified with another gene-specific primer, GSP2: 5'-CGATCACCTTTGTTGGATCC-3' (- 374 to - 355), combined with an (dC) adapter primer provided with the kit. This PCR product was used as a template for a nested PCR with another adapter AUAP provided with the kit and different specific primers of Tf2 transcript: (+66 to +47) 5'-ATCCAAATTCTCTGGTCTCC-3'; (-l34 to -l55) 5'-GGAGTTAATTGTAGATCACAAG-3'; (-53 to -73) 5'-TTCCTTCTCCTTTGAACCCAG-3'. Amplification on the complete LTR sequence was used to confirm the absence of DNA contamination: LTR forward primer 5'-TGTCAGCAATACTACACTACG-3' and reverse primer 5'-GTAAGCTACGCAGTTTGGT-3'. PCR products obtained by 5' RACE were isolated on agarose gel, cloned into pGEM-T (Invitrogen) and sequenced in both strands.

References

Allshire, R.C., Javerzat, J.P., Redhead, N.J. and Cranston, G. (1994) Position effect variegation at fission yeast centromeres. *Cell*, **76**, 157-169.

Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P. and Pringle, J.R. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. *Yeast*, **14**, 943-951.

Kurdistani, S.K., Robyr, D., Tavazoie, S. and Grunstein, M. (2002) Genome-wide binding map of the histone deacetylase Rpd3 in yeast. *Nat Genet*, **31**, 248-254.

Moreno, S., Klar, A. and Nurse, P. (1991) Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. *Methods Enzymol*, **194**, 795-823.

Robyr, D. and Grunstein, M. (2003) Genomewide histone acetylation microarrays. *Methods*, **31**, 83-89.

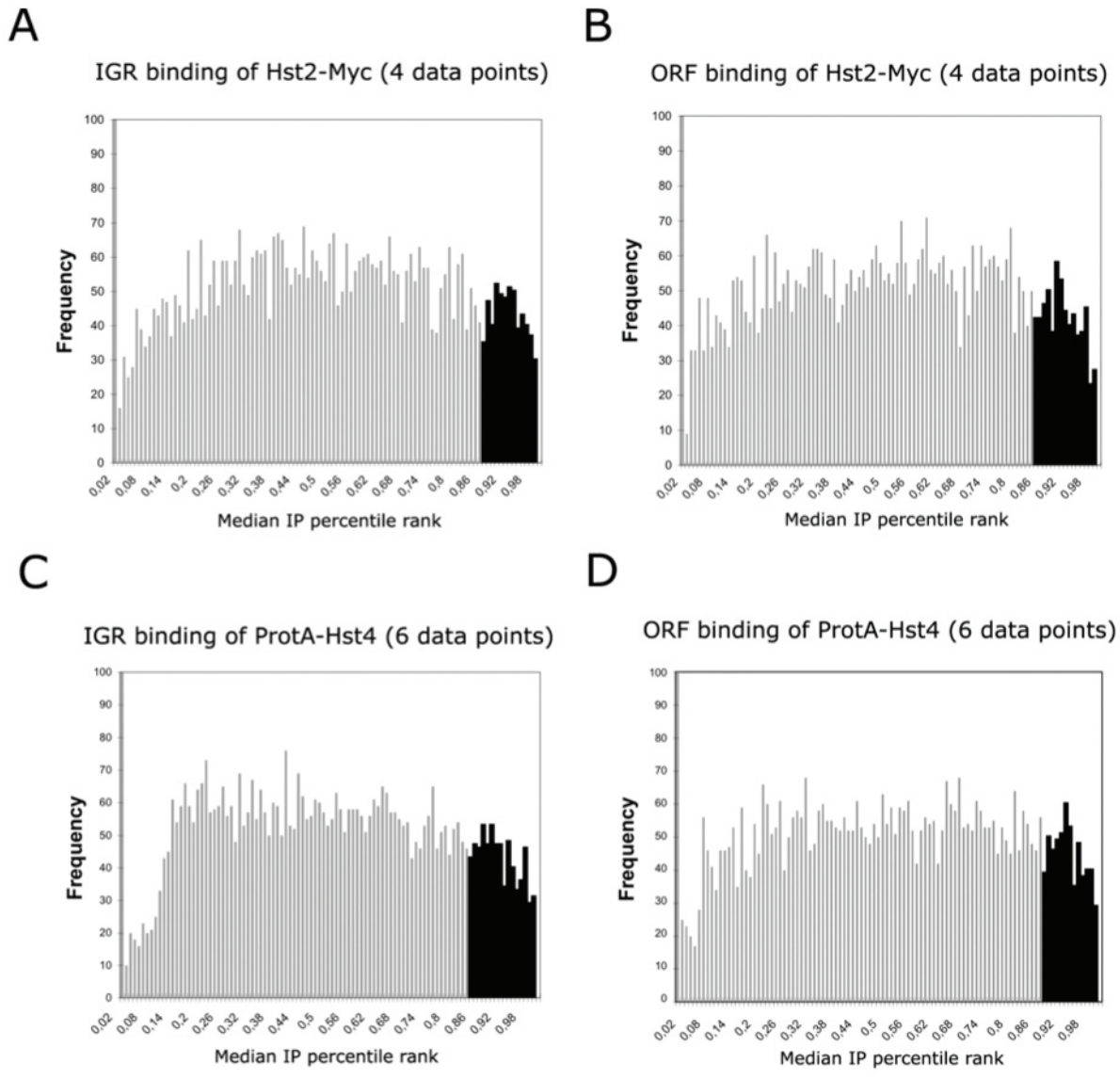
Suka, N., Luo, K. and Grunstein, M. (2002) Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat Genet*, **32**, 378-383.

Wiren, M., Silverstein, R.A., Sinha, I., Walfridsson, J., Lee, H.M., Laurenson, P., Pillus, L.,

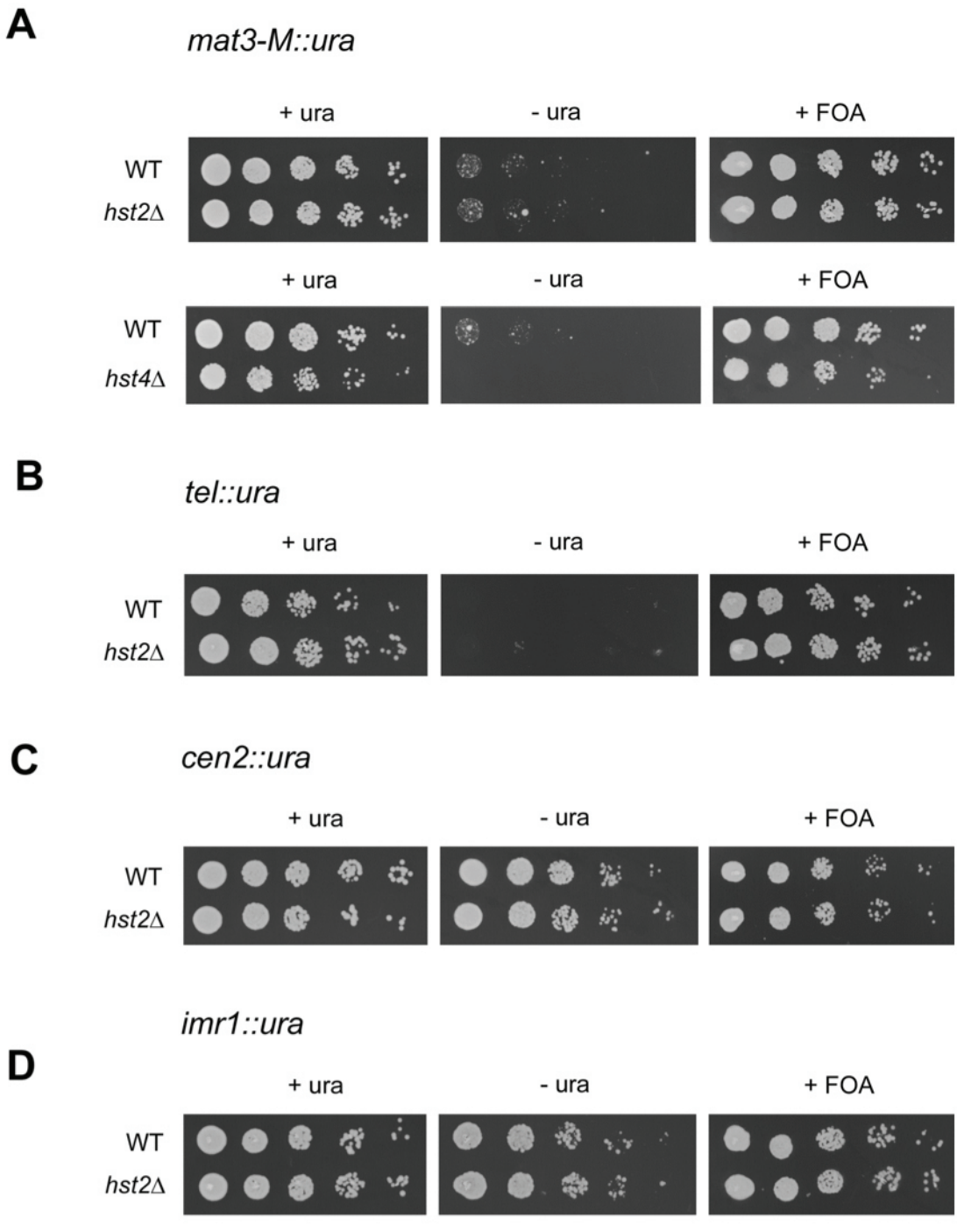
Robyr, D., Grunstein, M. and Ekwall, K. (2005) Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. *Embo J*, **24**, 2906-2918.

Xue, Y., Haas, S.A., Brino, L., Gusnanto, A., Reimers, M., Talibi, D., Vingron, M., Ekwall, K. and Wright, A.P. (2004) A DNA microarray for fission yeast: minimal changes in global gene expression after temperature shift. *Yeast*, **21**, 25-39.

Zeeberg, B.R., Feng, W., Wang, G., Wang, M.D., Fojo, A.T., Sunshine, M., Narasimhan, S., Kane, D.W., Reinhold, W.C., Lababidi, S., Bussey, K.J., Riss, J., Barrett, J.C. and Weinstein, J.N. (2003) GoMiner: a resource for biological interpretation of genomic and proteomic data. *Genome Biol*, **4**, R28.



Supplementary data S2 The median percentile ranking method was used to define statistical ChIP cutoff values for (A) IGR binding of Hst2-myc (M cut 0.88) (B) ORF binding of Hst2- myc (M cut 0.86) (C) IGR binding of ProtA-Hst4 (M cut 0.85) (D) ORF binding of ProtA-Hst4 (M cut 0.88). (A-D) The distribution of the median percentile rank for ChIP/INPUT (IP) samples is shown as a histogram. According to the median percentile ranking method to determine reproducible ChIP binding, the enriched fragments (data points) are constantly ranked high across the experimental replicates yielding an extra peak (black bars). Therefore, the median percentile 'M' cutoff for enriched fragments was defined as the beginning of the extra peak.



Supplementary data S5

Additional silencing assays of *hst2* and *hst4* (not shown in Figure 4). Fivefold dilutions of mid-exponential phase cells were plated onto EMM plates as a growth control (+ura), onto EMM plates lacking uracil (-ura) and onto 5-FOA plates (+FOA) to assay silencing of the *ura4+* reporter gene inserted in the *mat3-M* (A), *tel* (B), *cen2* (C), *imr1* (D). The strains used for these assays are described in Supplementary data S1.

A

ORF Binding Sir2-myc (M cut 0.84)R (n=582)	1,90E-23 (n=199) 8,01E-18 (n=224) 8,50E-05 (n=158) 0,00291 (n=145)	HIGH ORF *sir2*Δ H3K9Ac (n=900) HIGH ORF *sir2*Δ H4K16Ac (n=1178) HIGH ORF *sir2*Δ H4K5Ac (n=980) HIGH ORF *sir2*Δ H4K12Ac (n=921)
ORF Binding Hst2-myc (M cut 0.86)R (n=587)	1,50E-21 (n=175) 7,19E-16 (n=155) 2,99E-15 (n=146) 4,21E-11 (n=150) 4,50E-05 (n=140)	HIGH ORF *hst2*Δ H3K14Ac (n=765) HIGH ORF *hst2*Δ H4K5Ac (n=716) HIGH ORF *hst2*Δ H4K12Ac (n=666) HIGH ORF *hst2*Δ H4K16Ac (n=763) HIGH ORF *hst2*Δ H3K9Ac (n=828)
ORF Binding ProtA-Hst4 (M cut 0.88)R (n=543)	1,30E-17 (n=167) 1,59E-16 (n=156) 3,00E-16 (n=158) 1,22E-13 (n=156) 9,61E-13 (n=150)	HIGH ORF *hst4*Δ H3K9Ac (n=810) HIGH ORF *hst4*Δ H4K12Ac (n=749) HIGH ORF *hst4*Δ H4K16Ac (n=764) HIGH ORF *hst4*Δ H3K14Ac (n=799) HIGH ORF *hst4*Δ H4K5Ac (n=772)

B

IGR Binding Sir2-myc (M cut 0.91)R (n= 292)	1,32E-21 (n=112) 2,39E-21 (n=101) 8,99E-21 (n=108) 1,68E-20 (n=105) 4,72E-20 (n=101)	HIGH IGR *sir2*Δ H3K9Ac (n=618) HIGH IGR *sir2*Δ H4K12Ac (n=618) HIGH IGR *sir2*Δ H4K5Ac (n=624) HIGH IGR *sir2*Δ H4K16Ac (n=609) HIGH IGR *sir2*Δ H3K14Ac (n=575)
IGR Binding Hst2-myc (M cut 0.88)R (n= 529)	3,06E-27 (n=179) 3,69E-24 (n=176) 2,55E-22 (n=176) 3,03E-22 (n=178) 4,30E-16 (n=158)	HIGH IGR *hst2*Δ H3K14Ac (n=748) HIGH IGR *hst2*Δ H4K12Ac (n=698) HIGH IGR *hst2*Δ H4K5Ac (n=779) HIGH IGR *hst2*Δ H4K16Ac (n=798) HIGH IGR *hst2*Δ H3K9Ac (n=765)
IGR Binding ProtA-Hst4 (M cut 0.85)R (n=644)	7,89E-26 (n=180) 2,21E-20 (n=176) 3,59E-18 (n=160) 1,64E-14 (n=169) 1,59E-12 (n=162)	HIGH IGR *hst4*Δ H3K14Ac (n=700) HIGH IGR *hst4*Δ H4K12Ac (n=697) HIGH IGR *hst4*Δ H3K9Ac (n=665) HIGH IGR *hst4*Δ H4K16Ac (n=742) HIGH IGR *hst4*Δ H4K5Ac (n=757)

C

ORF Binding Sir2-myc (M cut 0.84)R (n=312)	1,25E-25 (n=157) 8,01E-05 (n=97) 2,99E-04 (n=91)	HIGH ORF *sir2*Δ H4K16Ac (n=1178) HIGH ORF *sir2*Δ H4K5Ac (n=980) HIGH ORF *sir2*Δ H4K12Ac (n=921)
ORF Binding Hst2-myc (M cut 0.86)R (n=284)	4,70E-04 (n=73)	HIGH ORF *hst2*Δ H3K9Ac (n=765)
ORF Binding ProtA-Hst4 (M cut 0.88)R (n=199)	0,00175 (n=56) 0,0206 (n=54)	HIGH ORF *hst4*Δ H4K12Ac (n=749) HIGH ORF *hst4*Δ H4K16Ac (n=764)

D

IGR Binding Sir2-myc (M cut 0.91)R (n= 76)	1,28E-06 (n=36)	HIGH IGR *sir2*Δ H4K12Ac (n=941)
IGR Binding Hst2-myc (M cut 0.88)R (n= 160)		
IGR Binding ProtA-Hst4 (M cut 0.85)R (n=239)	5,49E-07 (n=67) 4,49E-06 (n=63) 3,36E-05 (n=65) 5,32E-05 (n=61) 0,00109 (n=61)	HIGH IGR *hst4*Δ H3K14Ac (n=700) HIGH IGR *hst4*Δ H3K9Ac (n=665) HIGH IGR *hst4*Δ H4K16Ac (n=742) HIGH IGR *hst4*Δ H4K12Ac (n=697) HIGH IGR *hst4*Δ H4K5Ac (n=757)

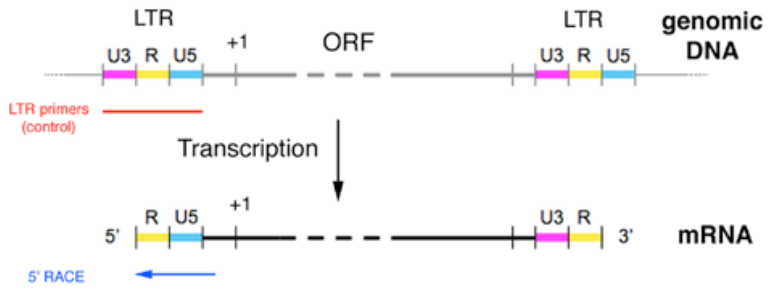
Supplementary data S6

Hypergeometric distribution comparisons of Sir2, Hst2, Hst4 binding data with histone acetylation changes in *sir2Δ*, *hst2Δ*, *hst4Δ*.

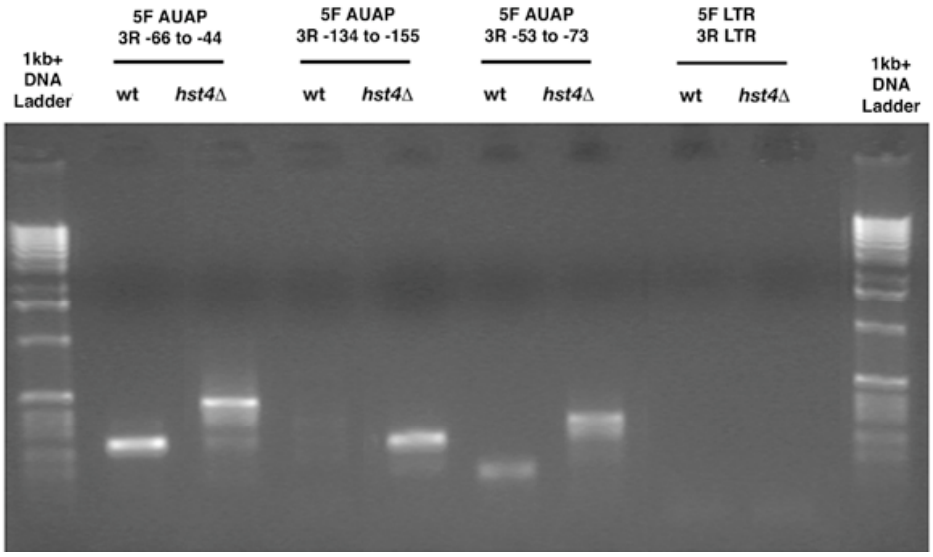
(A) ORF regions (B) IGR regions (C) Specific binding of each sirtuin at ORF regions (D) Specific binding of each sirtuin at IGR regions (A, B, C, D) Left column: Lists of fragment bound by Sir2, Hst2, and Hst4 (Percentile cut off values and number of bound fragments in each list are indicated).

Middle column: Hypergeometric P values and number of overlapping genes between binding and hyperacetylation in Sirtuin mutants. Right column: hyperacetylation in Sirtuin mutants (the histones acetylation sites and number of hyperacetylated fragments are indicated).

A



B



C

hst4Δ merged cDNA sequences (which correspond to the unprocessed mRNA transcript):

AGTTCAGTTATGAGCTATATTAGTGATAGGTAACATTATAACCCAGTTAATACAATA
CCTATACTCAGTTGCTACTTATACAACCTGTGTATTGTAATATAATAGATCACAAGGA
AAACTCACCGCAGTTCTACGTATCCTTAAATCAGATACCAAACTGCGTAGCTTACA**AT**
AACTGAACTCTTGTGATCTACAATTAACTCCTTTTAGGAAAAAGGAATTATTGAATA
AATATATATCATAAATATATATATTTCTCTCCTTCTGGGTTCAAAGGAGAAGGAGCTT
TGGAAGGACTGTTATCCCTTTTGAAATCTCCCAAAGGGAAACATATACA**ATG**TCCTAC
GCAAATTATCGTTATATGAAAGCAAGAGCAAAACGATGGAGACCAGAGAATTTGGAT

wt merged cDNA sequences (which correspond to the processed Tf2 transcript)

AACTCCTTTTAGGAAAAAGGAATTATTGAATAAATATATATCATAAATATATATATTT
CTCTCCTTCTGGGTTCAAAGGAGAAGGAACTTTGGAAGGACTGTTATCCCTTTTGAAA
TCTCCCAAAGGGAAACATATACA**ATG**TCCTACGCAAGTTATCGTTATATGAAAGCAA
GAGCAAAACGATGGAGACCAGAGAATTTGGAT

Supplementary data S7

5'-RACE reactions of total RNA from Hu1103 (*hst4Δ*) and Hu0303 (wt) strains.

(A) A representation of the genomic DNA and unprocessed mRNA transcript. 5' RACE of Tf2 RNA and control (LTR) primers are indicated. **(B)** A gel showing EtBr stained 5' RACE products obtained with different primers specific for the unprocessed Tf2 mRNA transcript. Lanes 2-7. A nested PCR was performed with the primer adapter AUAP and different specifics primers of Tf2 transcript : (-66 to -47) 5'-ATCCAAATTCTCTGGTCTCC-3'; (-l34 to -l55) 5'-GGAGTTAATTGTAGATCACAAG-3' ; (-53 to -73) 5'TTCCTTCTCCTTTGAACCCAG-3'. Lanes 9-10: Amplification of the complete LTR sequence was used to confirm the absence of DNA contamination.

(**C**) The merged cDNA sequences found with 5' RACE of Tf2 mRNA in *hst4*Δ and wt are represented. Highlighted nucleic acids in green are the theoretical Primer Binding-Site (PBS) and the complementary sequence. Repetitive (R) and U5 regions are respectively highlighted in yellow and blue.