Supplementary data:

1. **Materials and Methods** *(continued)*
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Materials and Methods (*continued from main text due to space limitation*)

Strains, genetic techniques and media

Standard fission yeast genetic techniques and media were used according to (Moreno et al., 1991). Cells were grown in rich YES medium. TSA (Sigma) was dissolved in DMSO and used at a final concentration in 60 μg/ml. The fission yeast strains Hu0303=972 *h-*, Hu0802 *h- clr6-1*, Hu1098 *h- sir2Δ::kanMX*, Hu1024 *h- clr3Δ::kanMX*, Hu1026 *h- hos2Δ::LEU2+ leu1-32* were used for cDNA expression profiling and the HDAC enzyme acetylation ChIP on CHIP experiments. Hu0619 *h90 clr3-myc::kanMX6 mat3-M::ura4+ ura4-DS/E leu1-32 ade6-M216,* LPY04586 *h- Sir2-myc::kanMX his3-D1 arg3-D4 ura1-D18leu1-32 ade6-216* were used for HDAC binding maps. Hu 427 h^{90} *clr3Δ::kanMX6 mat3-M::ura4*$^{+}$ *ura4-DS/E leu1-32 ade6-M210,*
Fy 597 h^{90}*mat3:: ura4*$^{+}$ $^{+}$ *ura4-DS/E leu1-32 ade6-M210, and* Hu 619 h^{90} *clr3-myc::kanMX6 mat3-M::ura4*$^{+}$ *ura4-DS/E leu1-32 ade6-M210* were used for spotting assays (Suppl Fig S3).

Expression profiling and ChiP on CHIP

Expression profiling was carried out according (Xue et al., 2004). We used the IGR and the IGR+ORF microarrays (produced at Eurogentec ASG, Belgium custom DNA microarray services) for Chromatin Immunoprecipitation (ChiP on CHIP). This combined IGR+ORF DNA microarray contains 124 fragments for extra coverage of long IGRs, and contains fragments of nearly all promoter regions and ORF regions. It therefore covers the genome with a 1.2 kb genome wide resolution (where 1.2 kb is the average distance between mid-points of the 500 bp fragments). For acetylation maps we used the ChiP on CHIP procedure described for genome wide histone acetylation maps by (Robyr and Grunstein, 2003) with following modifications. Cells were lysed by bead beating using a 'Biospec minibeadbeater' 4x40 sec at 4°C. We used specific antibodies for acetylation maps against acetylated isoform of histones H3K9Ac, H3K14Ac, H4K5Ac, H4K12Ac and H4K16Ac according to (Suka et

al., 2001). The histone H3 cter antibody was purchased from 'Upstate' and used for ChIP according to manufacturers recommendations. For HDAC binding maps we used the ChiP on CHIP procedure described by (Kurdistani et al., 2002) with following modifications. The ice cold PBS wash prior to fixation was omitted in some control experiments. Instead the cells were washed with PBS at room temperature. For Clr3-myc (strain Hu619) and Hos2-myc (Hu1135) crosslinking was carried out by treatment with formaldehyde for 30 min. Sir2-myc (Hu1131) was first treated with dimethyl adipimidate (DMA) for 45 min followed by crosslinking with formaldehyde for 6 hr. Cells were lysed using a 'Fastprep FP120' bead beater at speed setting 6.5, 5x25 sec at 4°C. The crude lysate was sonicated on ice 3x60 sec using a 'Branson sonifier 250', at settings: output control 7; 50% duty cycles. Cell debris was centrifuged 5 min at 20 000 rpm at 4°C, supernatant was transferred to a new fresh tube and kept on ice. The cell debris was resuspended in 400 µl lysis buffer, sonicated and centrifuged as described before. Supernatants from the two steps were then pooled. 1 µl of monoclonal anti-myc clone 9E10 antibody (Sigma) was used for ChIP. For Clr3-myc washing buffer containing 150 mM NaCl and for Sir2-myc washing buffer containing 500 mM NaCl were used. One example of the resulting DNA microarray after hybridization of ChIP DNA to the array is shown in Figure 1. Microarray scanners 'Agilent' or 'BIORAD' were used to generate Cy3 and Cy5 TIFF images that were quantified using Image Quant version 4.2 (Imagene). Spots were flagged according to the following quality criteria: empty spots threshold 0.8 and poor spots threshold 0.15. Three initial ChIP on CHIP array experiments we carried out dye swap controls (ChIP DNA labeled with Cy3 instead of Cy5), and these DNA microarray slides yielded surprisingly similar result with a correlation coefficient of 0.8-0.9 (Supplementary data Figure S1). Therefore we concluded that the Klenow labeling (Robyr and Grunstein, 2003) yields little dye bias and hence dye swaps were not routinely employed for the ChIP on CHIP experiments.

Microarray analysis and hypergeometric distribution tests

We have followed the guidelines of Microarray Gene Expression Data society (*www.mged.org/miame*.) and strictly maintained the minimum information about a microarray experiment (MIAME) format for all of our microarray experiments (Suppl. data Table 4). The data was imported into Gene Spring using the following data format: Separate measurements for replicate spots, median values for all signals, control channel and background measurements, and with flag values 0-present 2-absent 3-marginal (Imagene) or 3-present 2-

absent 0-marginal (Scanexpress). Ranking lists of nucleosome density (H3 cter) corrected IGR and ORF fragments with high histone acetylation levels as compared to wt were established and collected in database. High acetylation or expression in 2 of 2 (100%) in cases with only two data points, 3 of 4 (75%) in case of 4 data points, 4 of 6 (66%) in case of 6 data points for all acetylation profiling data. We use the same criteria for expression data except for TSA, *clr3Δ* and *clr6-1* datasets since we had so many more data points for these we otherwise get severe data loss due to flagging. By applying (4 of 8) (5 of 10) or (6 of 12) criteria for these more extensive data sets we compensate for data loss. Similar ranking lists were identified using the automatic hypergeometric distribution tests in the Gene List inspector function of gene Spring. The hypergeometric 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. The genomic physical position of the IGR and ORF fragments was visualized using map display function of gene Spring software.

References

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Tables:

Table 1

IGR Binding Sir2-myc 5 slides (M cut 0.91) 292 genes

P value	List Name
2,95e-240	**IGR Binding clr3-myc 5 slides (M cut 0.89)**
3,78e-36	**High IGR sir2 H3K9Ac H3 cter corr**
6,55e-33	High IGR sir2 H4K12Ac H3 cter corr
1,36e-32	High IGR sir2 H4K5Ac H3 cter corr
2,57e-31	High IGR sir2 H4K16Ac H3 cter corr
1,37e-30	High IGR sir2 H3K14Ac H3 cter corr
2,17e-22	High IGR hos2 H4K5Ac H3 cter corr
2,28e-21	High IGR hos2 H3K9Ac H3 cter corr
3,71e-21	high IGR hos2 H4K16Ac H3 cter corr
2,8e-20	High IGR hos2 H4K12Ac H3 cter corr
3,02e-12	High IGR clr3 H3K14Ac H3 cter corr
5,3e-12	High IGR hos2 H3K14Ac H3 cter corr
5,02e-10	High IGR clr3 H4K5Ac H3 cter corr
1,16e-7	High IGR clr3 H4K16Ac H3 cter corr
9,76e-7	High IGR clr3 H4K12Ac H3 cter corr
1,6e-6	High IGR clr3 H3K9Ac H3 cter corr
5,0e-5	sorbitol 15 min 2 fold up
0,0012	High IGR clr6-1 H4K5Ac H3 cter corr
0,00503	High IGR clr6-1 H4K16Ac H3 cter corr
0,00843	silent regions (cen, mat, tel, rDNA)
0,0418	High IGR clr6-1 H4k12Ac H3 cter corr

ORF Binding Sir2-myc 5 slides (M cut 0.84) 582 genes

P value	List Name
1,81e-153	**ORF Binding clr3-myc 5 slides (M cut 0.85)**
1,45e-55	High ORF hos2 H4K5Ac H3 cter corr
3,74e-54	High ORF hos2 H3K14Ac H3 cter corr
2,78e-52	High ORF hos2 H4K16Ac H3 cter corr
1,1e-46	High ORF hos2 H4K12Ac H3 cter corr
1,65e-45	High ORF hos2 H3K9Ac H3 cter corr
3,69e-30	**High ORF sir2 H3K9Ac H3 cter corr**
4,55e-15	High ORF clr3 H3K14Ac H3 cter corr
1,25e-14	High ORF clr3 H4K16Ac H3 cter corr
2,15e-9	HIGH expression in wild type norm ave (1-11)
4,11e-8	High ORF clr6-1 H3K14 H3 cter corr
7,93e-8	HIGH expression in clr6 (5 of 10) cut 1,5
4,13e-5	Component-Membrane
0,00253	stress >2 fold up
0,0133	wild type meiosis up >2 fold
0,0153	Cd 15 min 2 fold up
0,0179	Process-Transporters
0,0232	High IGR clr3 H4K5Ac H3 cter corr

Table 2

IGR Binding Clr3-myc 5 slides (M cut 0.89) 349 genes

P value	List Name
2,95e-240	**IGR Binding Sir2-myc 5 slides (M cut 0.91)**
9,32e-52	High IGR sir2 H3K9Ac H3 cter corr
5,12e-42	High IGR sir2 H4K5Ac H3 cter corr
5,91e-42	High IGR sir2 H4K12Ac H3 cter corr
4,03e-40	High IGR sir2 H4K16Ac H3 cter corr
4,41e-39	High IGR sir2 H3K14Ac H3 cter corr
1,47e-20	High IGR hos2 H4K5Ac H3 cter corr
6,95e-18	high IGR hos2 H4K16Ac H3 cter corr
1,5e-17	**High IGR clr3 H3K14Ac H3 cter corr**
5,44e-16	High IGR clr3 H4K5Ac H3 cter corr
1,09e-15	High IGR hos2 H4K12Ac H3 cter corr
1,74e-15	High IGR hos2 H3K9Ac H3 cter corr
5,06e-11	High IGR clr3 H4K16Ac H3 cter corr
1,26e-10	High IGR hos2 H3K14Ac H3 cter corr
1,29e-8	High IGR clr3 H4K12Ac H3 cter corr
3,31e-8	High IGR clr3 H3K9Ac H3 cter corr
6,16e-8	silent regions (cen, mat, tel, rDNA)
6,08e-5	High IGR clr3 H4K16Ac 3,0 fold embolast
6,7e-5	High IGR clr6-1 H4K5Ac H3 cter corr
0,0132	silent mat region
0,0294	High ORF clr3 H4K16Ac H3 cter corr

ORF Binding Clr3-myc 5 slides (M cut 0.85) 451 genes

P value	List Name
1,81e-153	**ORF Binding Sir2-myc 5 slides (M cut 0.84)**
1,95e-33	High ORF sir2 H3K9Ac H3 cter corr
2,52e-24	**High ORF clr3 H3K14Ac H3 cter corr**
3,6e-23	High ORF hos2 H4K16Ac H3 cter corr
3,8e-20	High ORF hos2 H4K5Ac H3 cter corr
4,03e-19	High ORF clr3 H4K16Ac H3 cter corr
9,67e-18	HIGH expression in wild type norm ave (1-11)
2,76e-13	High ORF hos2 H3K9Ac H3 cter corr
3,28e-13	High ORF hos2 H4K12Ac H3 cter corr
9,45e-13	High ORF hos2 H3K14Ac H3 cter corr
6,38e-7	High ORF clr6-1 H3K14 H3 cter corr
1,12e-5	HIGH expression in clr3 (4 of 8) cut 1,5
0,00383	HIGH expression in hos2 (3 of 4) cut 1,5
0,00939	HIGH expression in clr6 (5 of 10) cut 1,5
0,0206	High ORF clr6-1 H4K12Ac H3 cter corr
0,0248	HIGH expression in sir2 (4 of 6) cut 1,5
0,0345	Component-cell surface

Table 3

The effect of cold PBS wash in the Kurdistani et al ChIP protocol on binding to highly expressed genes

Hypergeometric distribution test of 'High expression in WT' (1048 genes)

P value	Gene list	Common
9,99e-14	ORF binding Clr3-myc RT (3 fold)	107/279
1,35e-10	ORF binding Clr3-myc ice cold (3 fold)	99/274
4,1e-8	ORF binding Sir2-myc RT (3fold)	119/381
0,000412	ORF binding Sir2-myc ice cold (3 fold)	173/693

Table 4 The MIAME format used

Array Design	Eurogentec SA ORF
	Eurogentec SA ORF+IGR
	Eurogentec SA IGR
Author	Karl Ekwall
Chip Batch	J230C; J130C; I240C etc.
Data processing/ normalization	Lowess (per spot per chip) or 50^{th} percentile (per chip)
Developmental Stage	mitosis
Drug/Small-molecule	TSA
Dye (signal)	Cy3; Cy5
Experiment Type	Treated vs. untreated
	H3 cter mut IP vs input
	H3 cter wt IP vs input
	Mutant vs wt
	IP vs input
Genetic Characteristic	*clr6-1*
	hos2Δ
	clr3Δ
	sir2Δ
Growth Conditions	Rich medium (YES)
Image Analysis Software	Imagene 4.1
	Imagene 4.2
	Scan express
	Quant Pro
Lab Book page	
Labeling Protocol	Xue et al (2004) Yeast:21:25-39 (expression profiling)

	Robyr et al (2003) Methods 31:83-89 (ChIP-CHIP) Kurdistani et al (2004) Nature Genetics 31:248-254 (HDAC binding)
Organism	*S. pombe*
Strain number	Hu802; Hu1098; Hu1024; Hu1026; Hu303 etc.
Temperature (deg-C)	25; 30; 36