Supporting Information

Materials and Methods

Plasmids. Plasmids are listed in Table S2. All clones were based on pLP1392, an *HST2* clone flanked by 400 bases of genomic sequence upstream and 300 bases downstream of the ORF in vector pRS313. 2μ constructs are based in pRS423. Truncation mutants were created by PCR sewing and confirmed by sequencing the full length of the clone. *HST2-NES*, *HST2*,* and *HST2*-NES* plasmids were constructed using site-directed mutagenesis (Wang and Malcolm, 1999). The *HST2-NES* lesions were specifically designed to maintain the sequence of helix α-13 (a.a. 303-314), while disrupting the two most highly conserved leucine residues in the NES, with the mutations L315A and L317A. The *HST2** lesion (H135Y) is directly analogous to the canonical, catalytically inactive *SIR2* allele, *sir2H364Y*. All clones have been verified to express the intended product as detected by protein immunoblotting with anti-Hst2 serum 663-4.

Dilution assays. For cycloheximide assays, YPD or synthetic medium lacking histidine was prepared using 250 ng/ml or 500 ng/ml cycloheximide (Sigma, St. Louis, MO) as a final concentration. For rDNA assays, synthetic medium lacking histidine or histidine and uracil was prepared (Sigma, St. Louis, MO). Cells were grown to saturation in liquid culture lacking drugs at 30˚C, and then plated as five-fold serial dilutions on solid media using a pinning tool. These plates were grown at 30˚C or 37˚C for up to 10 days, with digital images captured at two-day intervals.

Immunofluorescence microscopy. *hst2Δ* strain LPY6623 was transformed with plasmids bearing *HST2* or mutant constructs. Leptomycin B (KOSAN Biosciences, Inc., Hayward, CA), when used, was dissolved in 100% ethanol added to exponentially growing cultures at a final concentration of 100 ng/ml followed by a one-hour incubation at 30˚C.

Immunofluorescence was performed as described (Garcia and Pillus, 2002; Stone and Pillus, 1996) using a 1:200 dilution of 663-4 anti-Hst2 serum. Fluorescein-conjugated anti-rabbit secondary antibodies (Jackson Immunoresearch, West Grove, PA) were preadsorbed against spheroplasted yeast cells. Some cell-to-cell variability was observed in the intensity of Hst2 staining. This may be due to technical reasons such as variability in the degree of spheroplasting or for biological reasons such as differences in expression throughout the cell cycle. Microscopy was performed on an Applied Precision optical sectioning microscope to collect images spaced at 0.2-μm increments. The images were deconvolved using the Delta Vision deconvolution software as previously described (Rubio and Pogliano, 2004).

Yeast extracts. Strains were grown to log phase in liquid medium at 30˚C, then harvested by centrifugation. Pellets were resuspended at a density of 5 μl/OD600 in YEB-100 buffer (40 mM HEPES pH 7.5; 100 mM NaCl; 10% glycerol; 0.1% Tween-20) with protease inhibitors. Cells were lysed by vortexing with glass beads at 4˚C by four intervals of four minutes followed by two minutes on ice. Crude lysate was cleared by sedimenting five minutes at 14,000 rpm in a microfuge at 4˚C. For biochemical analysis, lysates were further cleared by two 30-minute microfuge runs at 14,000 rpm at 4˚C.

Chromatography. 50 μg total protein in 200 μl YEB-100 was fractionated over a Superdex 200 10/30 gel filtration column (Amersham Biosciences). 50 μl of each 250 μl fraction was assayed by immunoblotting.

Immunoblotting. Samples were subjected to electrophoresis on 9% acrylamide gels, and then transferred to nitrocellulose at 100V for 1 hour. Blots were pre-incubated with TBSTM (150 mM NaCl, 10 mM Tris, pH 8.0, 0.05% Tween 20, 2% powdered skim milk) for 15 minutes at room temperature. Cleared 663-4 anti-Hst2 serum was used as primary antiserum at a dilution

of 1:3000 in TBSTM, incubating at 4˚C overnight. Horseradish peroxidase-conjugated goat anti-rabbit secondary antiserum (Promega, Madison, WI) was used at a dilution of 1:10,000 for one hour at room temperature. Signal was developed using Western Lightning Chemiluminescence Reagent Plus (Perkin Elmer, Boston, MA) on X-OMAT film (Eastman Kodak Co., Rochester, NY).

Supplementary Table 1
Yeast strains used in this study.
All strains created for this study.

Strain	Genotype	Plasmid	Figure
LPY02446	*MATα his3Δ200 leu2Δ1 ura3-167 rDNA::mURA3*	none	
LPY06497	*MATa his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0*	none	3A
LPY06621	*MATa his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 hst2Δ::kanMX*	none	3A
LPY06623	*MATa his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 hst2Δ::kanMX*	none	
LPY08082	LPY06497	pLP60	3B
LPY08083	LPY06497	pLP1392	3B
LPY08085	LPY06497	pLP1720	3B
LPY08086	LPY06497	pLP359	3B, 3C,
LPY08087	LPY06497	pLP1723	3B, 3C,
LPY08089	LPY06497	pLP1721	3B
LPY08090	LPY06623	pLP60	S1
LPY08091	LPY06623	pLP1392	S1
LPY08092	LPY06623	pLP1695	S1
LPY08094	LPY06623	pLP359	2B
LPY08095	LPY06623	pLP1723	2B
LPY08097	LPY06623	pLP1721	2B
LPY10858	*MATa his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 crm1Δ::kanMX hst2Δ::natMX*	pLP1723, BT1602	1
LPY10860	*MATa his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 crm1Δ::kanMX hst2Δ::natMX*	pLP1723, BT1603	1
LPY10864	LPY06497	pLP1985	3B, 3C
LPY10865	LPY06497	pLP1986	3B
LPY10867	LPY06623	pLP1985	1
LPY11795	LPY06497	pLP2110	3C

LPY11796	LPY06497	pLP2111	3C
LPY11911	LPY02446	pLP359	3D
LPY11912	LPY02446	pLP1723	3D
LPY11913	LPY02446	pLP2110	3D
LPY11914	LPY02446	pLP1721	3D
LPY11915	LPY02446	pLP1985	3D
LPY11916	LPY02446	pLP2111	3D

Supplementary Table 2
Plasmids used in this study

.

Plasmid	Genotype	Source
pLP60	*HIS3 Amp^R CEN*	Sikorski and Hieter, 1989
pLP359	*HIS3 Amp^R 2μ*	Christianson, et al., 1992
pLP1392	*HST2 HIS3 Amp^R CEN*	this study
pLP1695	*hst2-ΔN6 HIS3 Amp^R CEN*	this study
pLP1720	*HST2-Δ298 HIS3 Amp^R CEN*	this study
pLP1721	*HST2-Δ298 HIS3 Amp^R 2μ*	this study
pLP1723	*HST2 HIS3 Amp^R 2μ*	this study
pLP1985	*HST2-NES HIS3 Amp^R 2μ*	this study
pLP1986	*HST2-NES HIS3 Amp^R CEN*	this study
pLP2110	*HST2* HIS3 Amp^R 2μ*	this study
pLP2111	*HST2*-NES HIS3 Amp^R 2μ*	this study
BT1602	*CRM1 LEU2 Amp^R CEN*	Neville and Rosbash, 1999
BT1603	*CRM1T539C LEU2 Amp^R CEN*	Neville and Rosbash, 1999

Figure S1. Hst2 appears largely monomeric and has a higher molecular weight form. Cleared yeast lysate containing 50 μg total protein was fractionated with Superdex 200 gel filtration. Hst2 was identified by immunoblotting with anti-Hst2 serum.

Accession numbers The GenBank accession numbers of proteins sequences discussed in this paper are: *Sc*Hst2 GI:6325242; *Hs*SIRT2 GI:24474785; *Mm*SIRT2 GI:38258618; *Rn*SIRT2 GI:56605812; *Xl*SIRT2 GI:55250553; *Dr*SIRT2 GI:68364282.

Supplementary References

Garcia, S.N. and Pillus, L. (2002) A unique class of conditional *sir2* mutants displays distinct silencing defects in *Saccharomyces cerevisiae*. *Genetics*, **162**, 721-736.

Rubio, A. and Pogliano, K. (2004) Septal localization of forespore membrane proteins during engulfment in *Bacillus subtilis*. *EMBO J*, **23**, 1636-1646.

Stone, E.M. and Pillus, L. (1996) Activation of an MAP kinase cascade leads to Sir3p hyperphosphorylation and strengthens transcriptional silencing. *J Cell Biol*, **135**, 571-583.

Wang, W. and Malcolm, B.A. (1999) Two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions using QuikChange Site-Directed Mutagenesis. *Biotechniques*, **26**, 680-682.

Zhao, K., Chai, X., Clements, A. and Marmorstein, R. (2003) Structure and autoregulation of the yeast Hst2 homolog of Sir2. *Nat Struct Biol*, **10**, 864-871.