SUPPLEMENTAL MATERIALS AND METHODS

Plasmid construction

Plasmids used in this study are listed in Table S1. The *sir2Δ::NatMX* deletion cassette in pLR809 was generated by homologous recombination in yeast. Specifically, the reading frame of *KlSIR2* in pLR730 (FROYD AND RUSCHE 2011) was replaced with NatMX amplified from pAGT100 (KAUFMANN AND PHILIPPSEN 2009) using primers 5'-tagctggaactggagcgcggaatattcattatctggagttcCCAGTGAATTCGAGCTCGG and 5'-atcagatcataagtgattcaaagcaacaagatttattcaaCATGATTACGCCAAGCTTGC. For the *K. lactis* mating assay, fluorescent proteins were cloned into integrating vector pGBN19 (READ *et al.* 2007), which drives expression from the *LAC4* promoter. First, the MFα1 leader sequence was removed from pGBN19 by digesting with *Hin*DIII and *Xho*I, blunting the ends, and religating the plasmid to generate pLR1076. Next, yeast enhanced GFP (yEGFP) was excised from pKT128 (SHEFF AND THORN 2004) using *Kpn*I and *Bam*HI and ligated into the *Kpn*I and *Bgl*II sites of pLR1076 to generate pLR1087. Separately, mCherry was excised from plasmid yEpGAP-cherry-MCS (KEPPLER-ROSS *et al.* 2008) using *Hin*DIII and *Bgl*II and ligated into the *Hin*DIII and *Bgl*II sites of pLR1076 to generate pLR1085.

Yeast strain construction

Yeast used in this study are listed in Table S2. Most *K. lactis* strains were derived from Os334 and Os335 (HEINISCH *et al.* 2010), which are congenic with the type strain CBS2359. To generate strains for RNA-Seq, we first deleted the *HM* loci. In strain Os334, *HMLα* was replaced with loxP-flanked KanMX from pCUG6 (PRIBYLOVA *et al.* 2007), and in strain Os335, *HMRa* was replaced with loxP-flanked *LEU2* from pJJ955L (HEINISCH *et al.* 2010). The markers were then removed by transiently transforming the yeast with pJJ958 (HEINISCH *et al.* 2010) expressing Cre recombinase and *URA3*. Next, these two strains

were crossed to generate LRY2835 with both *HM* loci deleted. Finally, repressor proteins were deleted

using the *sir2Δ::NatMX* deletion cassette from pLR809 to generate LRY2849 and 2850, the *sir4Δ::URA3*

cassette from LRY1946 (HICKMAN AND RUSCHE 2009) to generate LRY3096, or a *sum1Δ::KanMX* cassette

generated *in vitro* using the NEBuilder kit (New England Biolabs) and a KanMX cassette amplified from

pFA6a-KanMX (BAHLER *et al.* 1998) to generate LRY3098. To generate prototrophic strains (LRY2992,

LRY2993, LRY3027, and LRY3028), *ADE2*, *HIS3*, and *LEU2* were amplified from CK57-7A (CHEN AND CLARK-

WALKER 1994) and used to transform the RNA-Seq strains as well as an isogenic *MATα* strain derived

from the same cross that produced LRY2835. For the sporulation assay, diploid cells were generated by

mating haploid strains that were intermediates in the construction of prototrophic strains. *MATa ura3*

and *MATα leu2* haploids were mated to generate diploids homozygous for the deletions of the *HM* loci.

For the mating assay, the prototrophic strains were transformed with constructs to integrate

fluorescent proteins under the control of the *LAC4* promoter. *LAC4*::mCherry was derived from

pLR1085 cut with HpaI and XmaI, and *LAC4*::yEGFP was derived from pLR1087 cut with SacII. For the

ChIP-on-chip experiment, Sir2 was tagged as previously described (HICKMAN AND RUSCHE 2009) in strain

SAY538 (BARSOUM *et al.* 2010). The resulting strain was crossed to CK213-4c (KEGEL *et al.* 2006), and two

of the progeny, LRY2021 and 2022, were used for chromatin IP. Ambiguities were later noted in the

mating-type of LRY2022.

 S. cerevisiae strains were derived from the standard laboratory strain W303-1b. Most were

generated through transformations and crosses to recombine previously constructed alleles, including

hst1Δ::KanMX and *sir2Δ::TRP1* (RUSCHE AND RINE 2001), *HST1::5HA-URA3* (RUSCHE AND RINE 2001; HICKMAN

AND RUSCHE 2007), and *hmlαΔ::TRP1* (STONE *et al.* 2000). The *hmraΔ::URA3* allele was generated by one-

step gene replacement using *URA3* amplified from pRS406 with oligos 5'-

GAAATGCAAGGATTGGTGATGAGATAAGATAATGAAACATagattgtactgagagtgcac and 5'-

CCTCGAGGTGTAATCTAAATAATAACTTTATCGCAGTAGActgtgcggtatttcacaccg. The *SIR2::3HA-URA3* allele

was generated by one-step gene insertion at the end of the *SIR2* reading frame using a 3xHA tag

amplified from pLR522 (HANNER AND RUSCHE 2017) with primers 5'-

ATGGAAAAAGATTTTCAAGTGAATAAGGAGATAAAACCGTAT ggcggccgcatcttttac and 5'-

CAGGGTACACTTCGTTACTGGTCTTTTGTAGAATGATAAAgctcgaattcctgcagcccg.

Yeast transformation

   S. cerevisiae cells were transformed using the PEG-LiOAc method (SCHIESTL AND GIETZ 1989). Cells

were harvested at OD_{600} around 1 and washed twice with 0.1 volumes of TEL (10 mM tris, pH 7.5, 1

mM EDTA, 100 mM LiOAc). Cells were resuspended in TEL at 10 µl/OD cells, and 100 µl of cells were

added to 0.1 µg of linear DNA plus 30 µg sheared salmon sperm DNA. Cells were incubated at 30° for

30 minutes, combined with 750 µl 40% PEG-TEL, and incubated at 30° for 30 minutes. Finally, cells

were heat shocked at 42° for 10 minutes and plated on selective medium. *K. lactis* cells were

transformed using electroporation (HICKMAN AND RUSCHE 2009). Briefly, cells were harvested at an

optical density around 1 and resuspended at 15 OD/ml in YPD containing 25 mM DTT and 20mM

HEPES, pH 8. Cells were shaken for 30 minutes at 30°, collected, and washed in electroporation buffer

(10mM tris pH7.5, 270mM sucrose, 1mM LiOAc). Cells were then resuspended at 100 OD/ml in

electroporation buffer. Electroporation reactions were set up in 0.2 mm cuvettes using 50 µL cells, 1

µL 10 mg/mL salmon sperm DNA, and 0.5-1 µg DNA in a volume no more than 5 µL. Electroporation

conditions were 1000 V, 300 Ω, and 25 µF. After electroporation, cells were incubated in YPD four

hours at 30° and then spread on selective media.

Chromatin IP and processing for microarray or sequencing

For the ChIP on Chip experiment from *K. lactis*, chromatin IP was conducted as previously described (HICKMAN AND RUSCHE 2009), with some exceptions. Cells were crosslinked for one hour each in 10 mM DMA and then 1% formaldehyde. After cell lysis, chromatin was sonicated four times for 15 seconds. 160 µl of lysate (derived from 10 OD equivalents of cells) was brought to a final volume of 400 µl in lysis buffer and incubated overnight with 7 µl anti-HA antibody (Upstate). The immunoprecipitated DNA was labeled with either Cy5- or Cy3-conjugated dUTP (Perkin Elmer NEL578001EA or NEL579001EA), using Klenow DNA polymerase (NEB M0212M) and random nonamer oligonucleotides (IDT). 500 ng of input DNA or an entire immuno-precipitated DNA sample was dried in a speed-vac and resuspended in 15 µL of primer mix (1X NEB buffer 2, 5 µg of random nonamer). Once the DNA was dissolved, 2 nmole of labeled dUTP was added in 2 µl. The samples were placed in a thermocycler and denatured for 5 minutes at 95°, and then cooled to 4°. The samples were combined with 3 µL of Klenow reaction mix, resulting in a final concentration of 1X NEB buffer 2, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.1 mM dTTP and 12.5 U of Klenow. The sample was ramped to 37° at 0.1°/sec and then incubated for 30 minutes. Following incubation, the sample was heat-denatured and cooled to 4°, and fresh Klenow (4 U) was added for a second round of labeling. Finally, unincorporated nucleotides, oligonucleotides, and dye were removed using Microcon YM-30 filters (Millipore). Labeled DNA was hybridized to the tiled Agilent array in hybridization buffer overnight at 65°. The microarray was washed and scanned according the manufacturer's instructions.

For the ChIP-Seq experiment from *S. cerevisiae,* chromatin IP was performed essentially as described (RUSCHE AND RINE 2001). Cells were harvested at OD_{600} around 1. Cells were crosslinked for one hour each in 10 mM DMA and then 1% formaldehyde. The immunoprecipitation was conducted with 10µL of Protein A agarose beads in the absences of BSA and salmon sperm DNA. Library

preparation and sample barcoding was done at the Next-Generation Sequencing facility at University at Buffalo. The samples were then sequenced on an Illumina HiSeq2500 using 50 bp single-end sequencing.

For gene by gene analysis (Figure S3), chromatin IP was performed essentially as described (RUSCHE AND RINE 2001). Cells were harvested at OD_{600} around 1. For KlSir2-HA or ScHst1-HA ChIP, cells were crosslinked 30 minutes in 10 mM DMA and then 30 minutes in 1% formaldehyde. For histone ChIP, cells were crosslinked for 20 minutes in 1% formaldehyde. Antibodies used were anti-HA tag (Sigma H6908), anti-H3 (Millipore 05-928), and anti-H3K9^{Ac} (06-942). For each gene of interest, the enrichment of the promoter was compared to a control locus, *MSH5*, using primers listed in Table S11. *MSH5* is distant from a Hst1 or Sir2 peak (40 kb to ScHst1, 53 kb to ScSir2, 17 kb to KlSir2), so it represents background recovery of genomic DNA. In addition, *MSH5* is a long gene (2.7 kb in *S. cerevisiae*, 2.8 kb in *K. lactis*) that is lowly expressed in both species. Thus, histone H3 should be relatively unacetylated in the center of the gene. PCR amplicons corresponding to the promoters and control locus were quantified by qPCR relative to a standard curve prepared from ChIP input DNA. Then, for each IP sample, the relative enrichment was calculated as the ratio of the promoter to the control locus. For KlSir2-HA or ScHst1-HA ChIP, four independent IP's were conducted from four separate cultures of each strain. For histone ChIP, the ratio of H3K9^{Ac} to total H3 was calculated by dividing the relative enrichments of the H3K9^{Ac} and H3 IPs from the same culture. Five or six independent IP's were conducted with each antibody from independent cultures of each strain.

cDNA synthesis and quantification

For gene by gene analysis (Figure S3), RNA was isolated as previously described (HANNER AND RUSCHE 2017). DNA was removed from 3 μg RNA using Optizyme DNase I (Fisher BioReagents) in the

manufacturer's buffer for 30 minutes at 37°. The sample was then extracted with phenol/CHCl$_3$,

precipitated with ethanol, and resuspended in 30 µl H$_2$O. Treated and untreated samples were

compared by qPCR to confirm that the DNase reaction was successful. cDNA was synthesized using

iScript Advanced reverse transcriptase according to the manufacturer's instructions. The reaction was

diluted ten-fold and quantified by qPCR using primers listed in Table S11 and a standard curve

prepared from genomic DNA. For each sample, the relative expression was calculated as the ratio of

the gene of interest to the control, *CWC15*, a gene of medium expression that is not affected by the

deletion of *SIR2* or *HST1* in either *S. cerevisiae* or *K. lactis*.

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LIST OF TABLES

Tables S1 and S2 are included in this document. Tables S3-S9 are posted on FigShare.

Table S1. Plasmids used in this study

Table S2. Yeast strains used in this study

Table S3. Mapping percentages for Illumina sequencing experiments

Table S4. RNA-Seq and ChIP-Seq data for all *S. cerevisiae* genes

Raw data for all annotated *S. cerevisiae* genes.

Table S5. RNA-Seq and ChIP-Chip data for all *K. lactis* genes

Raw data for all annotated *K. lactis* genes.

Table S6. ScSir2-regulated genes

Each row represents a gene that was both associated with ScSir2 and upregulated at least two-fold in *sir2Δ hst1Δ* compared to wild-type *S. cerevisiae*. The KlSir2-regulated column indicates whether the *K. lactis* ortholog is regulated by KlSir2 based on both RNA-Seq datasets (12&16) or just the newer dataset (2016). The Ellahi column indicates whether the gene was identified by (ELLAHI *et al.* 2015) as SIR-regulated.

Table S7. ScHst1-regulated genes

Each row represents a gene that was both associated with ScHst1 and upregulated at least two-fold in *sir2Δ hst1Δ* compared to wild-type *S. cerevisiae*. The KlSir2-regulated column indicates whether the *K. lactis* ortholog is regulated by KlSir2 based on both RNA-Seq datasets (12&16) or just the older dataset (2012). The Bedalov column indicates whether the gene was identified by (BEDALOV *et al.* 2003) as Hst1-regulated. The McCord columns indicate whether the gene was identified by (MCCORD *et al.* 2003) as Hst1- or Sum1-regulated. The Borde and Friedlander columns indicate whether the gene was increased during sporulation in two expression studies (FRIEDLANDER *et al.* 2006; BORDE *et al.* 2009). The categories and subcategories were developed manually based on GO terms and functional information about each gene.

Table S8. KlSir2-regulated genes identified using 2016 RNA-Seq data

Each row represents a gene that was both associated with KlSir2 and upregulated in the 2016 dataset at least two-fold in *sir2Δ* compared to wild-type *K. lactis*. The *S. cerevisiae* orthologs were determined through a reciprocal BLASTP procedure followed by manual refinement, as described in the methods. For genes whose top *S. cerevisiae* BLASTP hit was more similar to another *K. lactis* gene, no *S. cerevisiae* ortholog is given. Instead, the description indicates that the gene is related to its top hit. The 2012 column indicates whether the gene was also induced in the 2012 RNA-Seq dataset. The categories and subcategories were developed manually based on GO terms and functional information about each gene and its *S. cerevisiae* ortholog.

Table S9. KlSir2-regulated genes identified using 2012 RNA-Seq data

Each row represents a gene that was both associated with KlSir2 and upregulated in the 2012 dataset at least two-fold in *sir2Δ* compared to wild-type *K. lactis*. Genes that were also upregulated in the 2016 dataset are excluded from this list and can be found in Table S7. Columns are as described for Table S7.

Table S10. RNA-Seq and ChIP-Seq data for metabolic genes

This table is the basis for Figure 3 and includes all *S. cerevisiae* genes known to act in the each pathway included in the figure. For each gene and its *K. lactis* ortholog, data are provided for the association with ScSir2, ScHst1, and KlSir2 and the expression change in deletion compared to wild-type cells.

Table S11. Oligonucleotides used for qPCR