Figure S1
(A) Distrubtion of the $\log_2$ of IP/input for the Sir2-HA ChIP from LRY 2021. The long tail to the right indicates enrichment in IP channel.
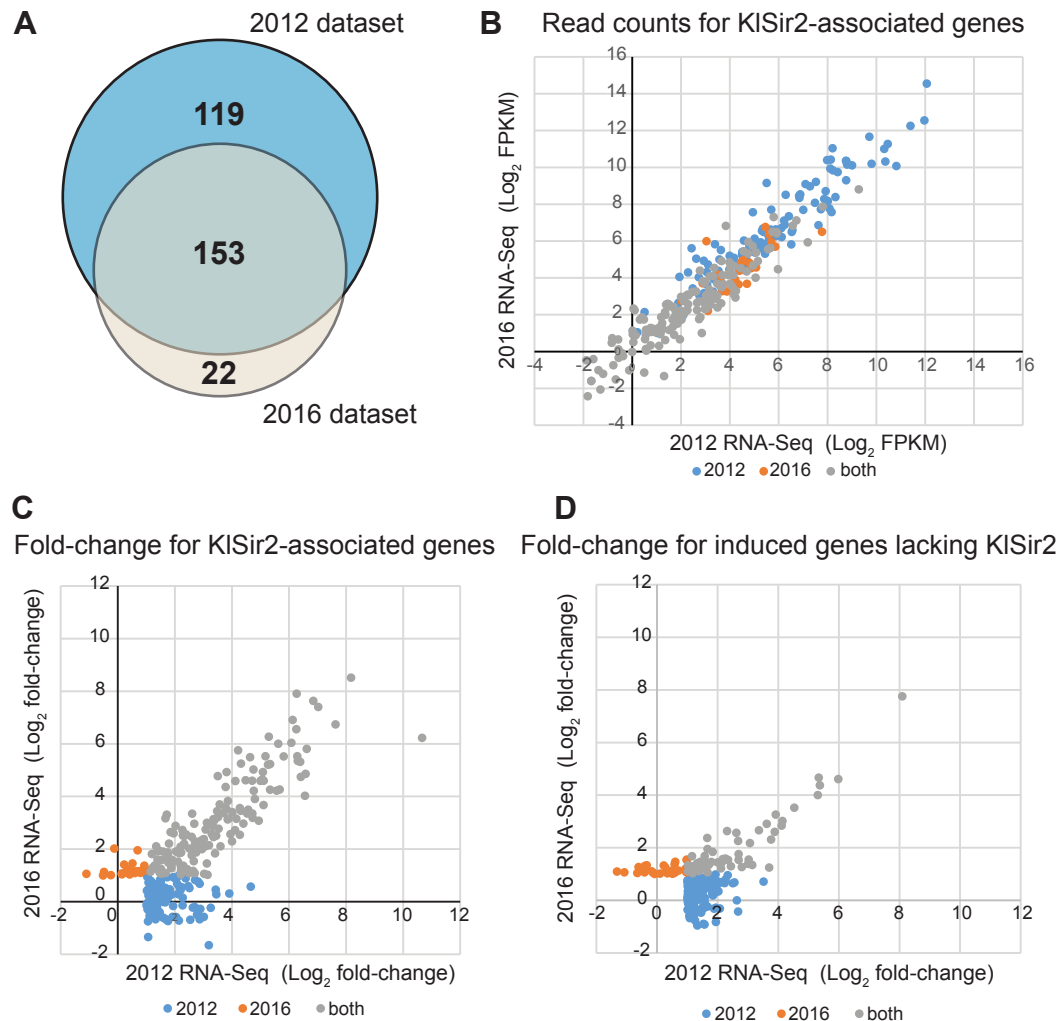(B) Distrubtion of the $\log_2$ of IP/input for the Sir2-HA ChIP from LRY 2022.

Figure S2. Comparison of two *K. lactis* RNA-Seq datasets.
(A) The overlap was determined for KlSir2-associated genes induced in *sir2Δ* compared to wild-type cells in the 2012 RNA-Seq dataset (272) and the 2016 dataset (175).
(B) For each KlSir2-associated gene induced in at least one dataset, the $\log_2$ FPKM (Fragments Per Kilobase of transcript per Million mapped reads) from wild-type cells from each dataset was plotted. Genes that were induced in both datasets (gray) tended to have lower expression (FPKM) than genes induced in only one of the two datasets (2012, blue or 2016, red).
(C) For each KlSir2-associated gene induced in at least one dataset, the $\log_2$ fold-change (*sir2Δ*/wild-type) from each dataset was plotted. Genes that were induced in both datasets (gray) tended to have higher fold-change than genes induced in only one of the two datasets.
(D) For induced genes that were not associated with KlSir2, the $\log_2$ fold-change (*sir2Δ*/wild-type) from each dataset was plotted. Fewer of these genes were induced in both datasets.
These data strengthen the conclusion that the 153 KlSir2-associated genes induced in both datasets are repressed by KlSir2.
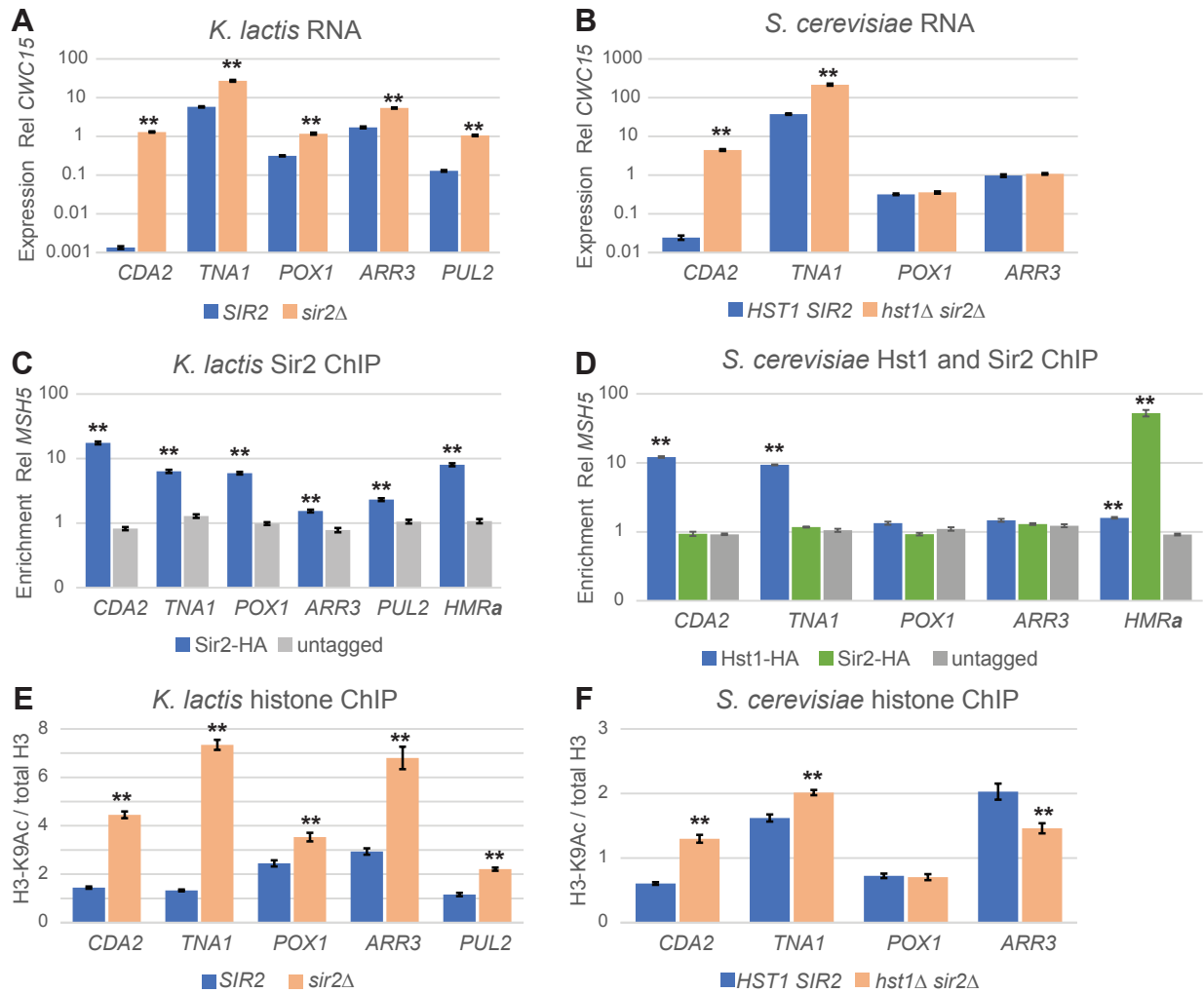
Figure S3. Analysis of individual genes was consistent with genome-wide data.
(A) RNA was extracted from *SIR2* (LRY2992) and *sir2Δ* (LRY2993) *K. lactis* cells, converted to cDNA, and quantified by qPCR. The amount of each mRNA was normalized to Kl*CWC15*, which is not regulated by KlSir2. Error bars represent the standard deviation based on five independent RNA isolations. ** indicates p value < 0.01 of *sir2Δ* compared to *SIR2* cells using a two-tailed t-test.
(B) RNA was extracted from *HST1 SIR2* (LRY3093) and *hst1Δ sir2Δ* (LRY3099) *S. cerevisiae* cells and analyzed as for part A. Sc*CWC15* is not regulated by ScHst1 or ScSir2. Error bars represent the standard deviation of six independent RNA isolations. ** indicates p value < 0.01 of *hst1Δ sir2Δ* compared to *HST1 SIR2* cells using a two-tailed t-test.
(C) Chromatin IP using HA antibody of *SIR2-HA* (LRY2021) and untagged (LRY1506) *K. lactis* cells. The enrichment of the promoter of each gene was normalized to the center of Kl*MSH5*, a long, lowly expressed gene that is not near a KlSir2 peak. Error bars are based on four independent IP's. ** indicates p value < 0.01 for tagged strain compared to untagged strain based on a two-tailed t-test. *HMRa* is a mating-type locus repressed by Sir proteins.
(D) Chromatin IP using HA antibody of *HST1-HA* (LRY558), *SIR2-HA* (LRY1926) and untagged (LRY1009) *S. cerevisiae* cells. Analysis was conducted as for part C. Sc*MSH5* is lowly expressed and not near ScHst1 or ScSir2 peaks. Error bars are based on four independent IP's. ** indicates p value < 0.01 for tagged strain compared to untagged strain based on a two-tailed t-test.
(E) Chromatin IP using antibodies against acetylated H3K9 and total H3 of *SIR2* (LRY2992) and *sir2Δ* (LRY2993) *K. lactis* cells. The enrichment of the promoter of each gene was normalized to Kl*MSH5* for each IP, and then the ratio of H3-K9Ac to total H3 was determined. Error bars are based on six independent IPs. ** indicates p value < 0.01 of *sir2Δ* compared to *SIR2* cells using a two-tailed t-test.
(F) Chromatin IP using antibodies against acetylated H3K9 and total H3 of *HST1 SIR2* (LRY3093) and *hst1Δ sir2Δ* (LRY3099) *S. cerevisiae* cells. The ratio of H3-K9Ac to total H3 was determined as for part E. Error bars are based on five independent IPs. ** indicates p value < 0.01 of *hst1Δ sir2Δ* compared to *HST1 SIR2* cells using a two-tailed t-test. Although there is a significant change in acetylation of the Sc*ARR3* promoter, it is in the opposite direction expected for the loss of a deacetylase.
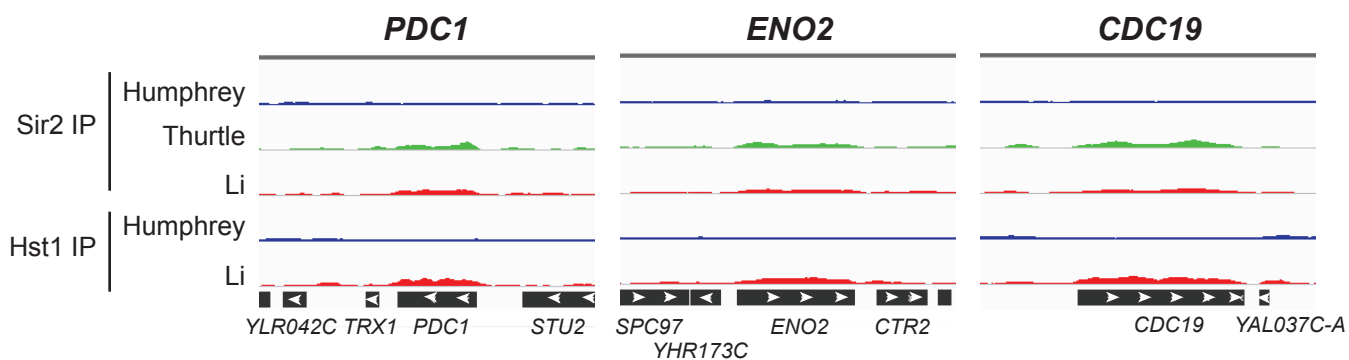
Figure S4. ScSir2 and ScHst1 were not enriched at highly expressed genes.
The read depth of ScSir2 and ScHst1 ChIP-Seq sequencing reads from this study (Humphrey) and two others (Thurtle and Rine, 2014; Li et al., 2013) are shown at three genomic loci reported to be associated with these deacetylases (Li et al., 2013). Our data revealed little enrichment of ScSir2 or ScHst1. In the other studies, slight enrichment was observed, but could result from a known "hyper-ChIP" artifact (Teytelman et al., 2013).
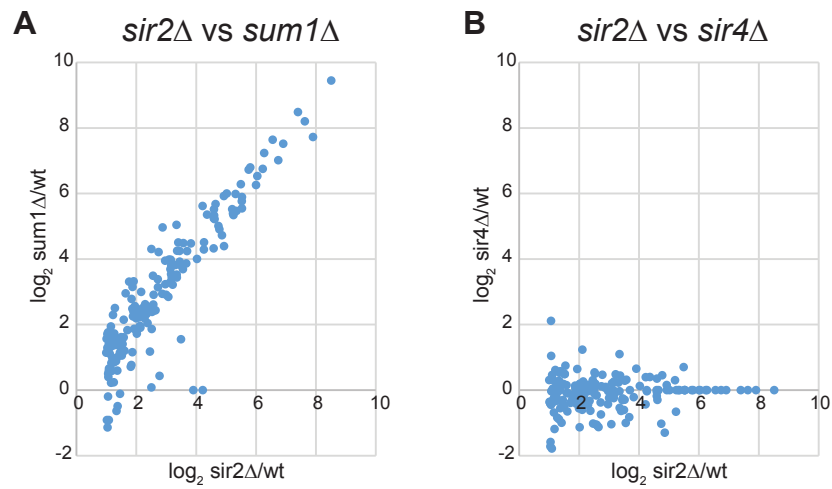
A *sir2Δ* vs *sum1Δ*

B *sir2Δ* vs *sir4Δ*

Figure S5. Most KlSir2-regulated genes were also regulated by KlSum1.
(A) For each KlSir2-regulated gene, the $\log_2$ fold-change was plotted for *sir2Δ* compared to wildtype cells and *sum1Δ* compared to wildtype cells. Expression is correlated in the two strains.
(B) For the same genes, the $\log_2$ fold-change was plotted for *sir2Δ* compared to wildtype cells and *sir4Δ* compared to wildtype cells. Expression is not correlated in the two strains.
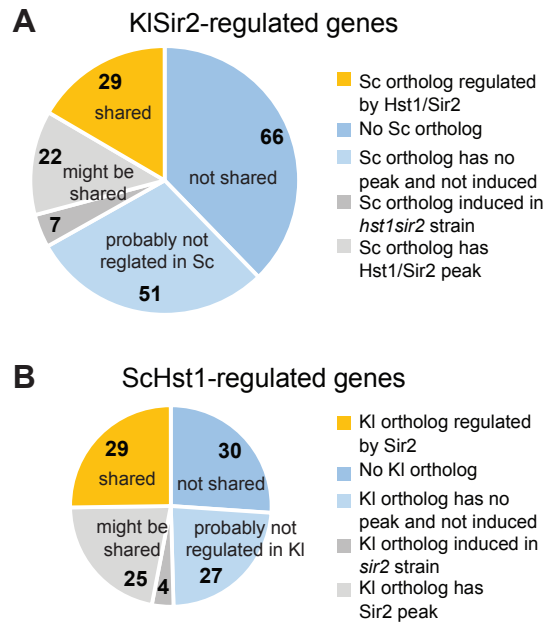
A KlSir2-regulated genes

29 shared
66 not shared
22 might be shared
7
probably not reglated in Sc
51

- Sc ortholog regulated by Hst1/Sir2
- No Sc ortholog
- Sc ortholog has no peak and not induced
- Sc ortholog induced in *hst1sir2* strain
- Sc ortholog has Hst1/Sir2 peak

B ScHst1-regulated genes

29 shared
30 not shared
might be shared
probably not regulated in Kl
25 4 27

- Kl ortholog regulated by Sir2
- No Kl ortholog
- Kl ortholog has no peak and not induced
- Kl ortholog induced in *sir2* strain
- Kl ortholog has Sir2 peak

Figure S6. More than half of genes repressed by KlSir2 and ScHst1 were only regulated in one of the two species.

(A) For KlSir2-regulated genes, 67% (117/175) are likely not regulated in *S. cerevisiae*, either because there is no *S. cerevisiae* ortholog (66) or because the ortholog did not meet either of the criteria for regulation (51; criteria are association of ScHst1 and change of expression in *hst1Δ sir2Δ* strain). Of the remaining genes, 16.5% (29/175) were not identified as ScHst1-regulated, but did meet one of the criteria.
(B) For ScHst1-regulated genes, 49.5% (57/115) are likely not regulated in *K. lactis*, either because there is no *K. lactis* ortholog (30) or because the ortholog did not meet either of the criteria for regulation (27). Of the remaining genes, 25% (29/115) were not identified as KlSir2-regulated, but did meet one of the criteria.