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Supplemental Data

Bypassing Sir2 and *O*-Acetyl-ADP-Ribose in Transcriptional Silencing

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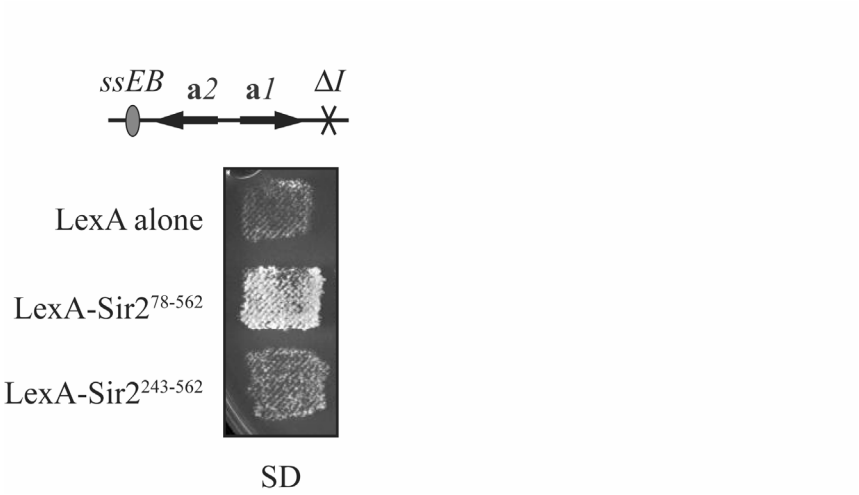

Figure S1 – LexA-Sir2$^{243\text{-}562}$ does not impart targeted silencing. Previous work showed that tethering Sir proteins to DNA can nucleate silent chromatin, which spreads and represses nearby genes (Chien et al., 1993; Cuperus et al., 2000; Cockell et al., 2000). Strain YCL73 (*MATα Δhmr::6lex^{ops}ssEB-a2a1 Δsir2 Δsir1*) with LexA operators (*lex^{ops}*) at a synthetic *HMR-E* silencer (labeled *ssEB,* (Li et al., 2001)) was patch mated with the K125 tester strain. The strain was transformed with plasmids that express Sir2$^{243\text{-}562}$ (pCC18), LexA fused to either Sir2$^{78\text{-}562}$ (pCC27), or LexA alone (pJK1521). LexA-Sir2$^{78\text{-}562}$ produced robust targeted silencing (also see figure 3C). LexA-Sir2$^{243\text{-}562}$ and LexA alone did not silence at all.

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Figure S2

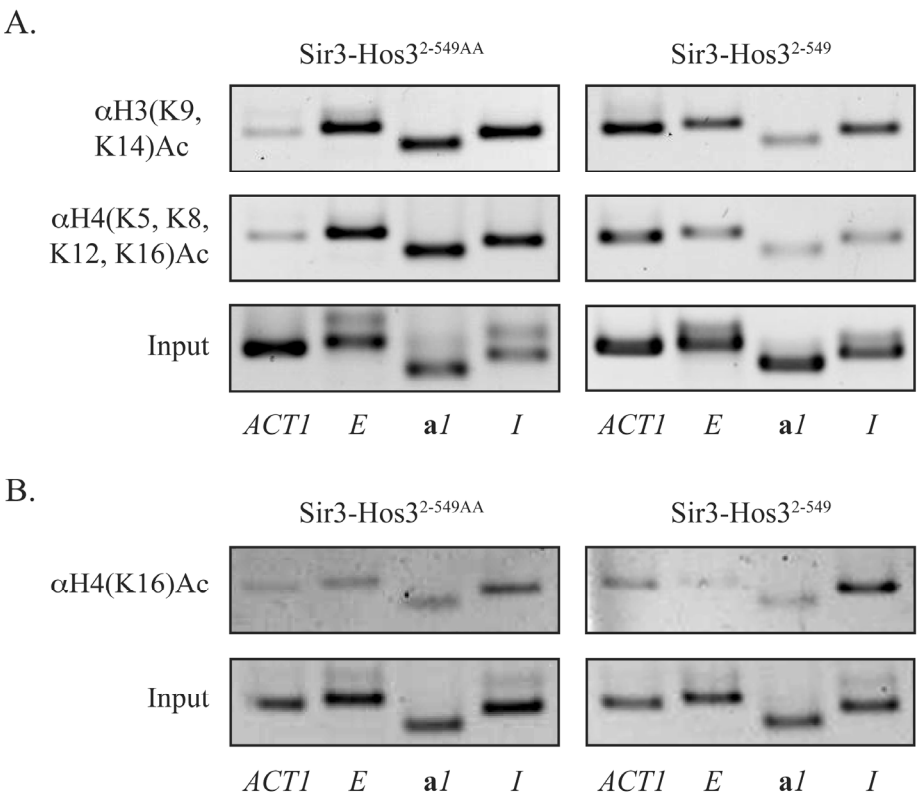

Figure S2 – Representative examples of raw ChIP data. Extracts were prepared from the strains described in Figure 4. **A)** PCR reactions of Input and samples immunoprecipitated with either anti-H3(K9, K14)Ac or anti-H4(K5, K8, K12, K16)Ac. **B)** PCR reactions of Input and samples immunoprecipitated with anti-H4K16Ac.

Table S1. Strains

Name	Genotype	Reference
K125	*MAT***a** *hom3*	(Tatchell et al., 1981)
K126	*MATα hom3 ilv1*	“”
YCB496	*MATα ura3-52 his3Δ200 leu2Δ1 trp1Δ63 ade2-101 lys2-801 hst1Δ2::LEU2 hst2Δ1::TRP1 hst3Δ3::HIS3 hst4Δ1::URA3 sir2Δ1::URA3*	(Brachmann et al., 1995)
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(Thomas and Rothstein, 1989)
GCY16	W303-1A *Δsir2::kanMX*	(Cuperus et al., 2000)
YCL73	W303-1A *MATα hmr::RS-6lex^{ops}ssEB-***a***2***a***1-RS Δlys2 Δsir2::klURA3 Δsir1::loxP-kanMX-loxP Δbar1::hisG*	(Li et al., 2001)
YDS631	W303-1A *MATα adh4::URA3::C$_{1-3}$A*	(Chien et al., 1993)
GA2050	W303-1A *MATα Aeb4lex^{ops}::TRP1::HMR-I*	(Taddei et al., 2004)
JN19	GA2050 *Aeb4lex^{ops}::klURA3::HMR-I*	This study
CCC1	YDS631 *MATα Δsir2::kanMX*	“”
CCC8	GCY16 *MAT***a** *Δsir2::kanMX Δsir3::HIS3*	“”
CCC11	YDS631 *Δsir3::HIS3*	“”
CCC12	YDS631 *MATα Δsir2::kanMX Δsir3::HIS3*	“”
CCC18	GCY16 *MAT***a** *Δsir2::hphMX Δsir3::HIS3*	“”
CCC19	YDS631 *MATα Δsir2::hphMX Δsir3::HIS3*	“”
CCC20	GCY16 *MAT***a** *Δsir2::kanMX Δsir3::HIS3 Δsir4::hphMX*	“”
CCC21	YDS631 *MATα Δsir2::kanMX Δsir3::HIS3 Δsir4::hphMX*	“”
CCC37	YDS631 *MATα Δsir2::hphMX Δsir3::HIS3 Δhst2::kanMX*	“”
CCC38	GCY16 *MAT***a** *Δsir2::hphMX Δsir3::HIS3 Δhst2::kanMX*	“”
CCC44	JN19 *Δsir2::hphMX*	“”
CCC45	GCY16 *MAT***a** *Δsir2::hphMX Δsir3:HIS3 Δhst1::kanMX*	“”
CCC46	YDS631 *MATα Δsir2::hphMX Δsir3::HIS3 Δhst1::kanMX*	“”
CCC48	*MATα Δsir2::hphMX Δsir3::HIS3 Δhst1::kanMX*	“”
CCC50	CCC48 *MATα Δsir2::hphMX Δsir3::HIS3 Δhst1::kanMXΔhst2::loxP-klURA3-loxP*	“”

All strains were derived from W303-1A, except K125 and K126 and the S288C-derived strain YCB496.

Table S2. Expression vectors

Name	Marker	Description	Primers[†]	Construction
pSIR3-HA^{TRP1}	*TRP1*	Sir3^{3xHA}	-	*TRP1* version of pSIR3-HA (Ansari and Gartenberg, 1999).
pBTM116[#]	*TRP1*	LexA	-	(Bartel and Fields, 1995)
pGLC117[#]	*TRP1*	LexA-Sir2$^{78-562}$	-	(Cuperus et al., 2000)
pJK1521[#]	*TRP1*	LexA alone	-	(Kamens et al., 1990)
pCC1	*TRP1*	Sir3-Sir2$^{2-562}$	1, 2	Terminal 3xHA epitopes of pSIR3-HA^{TRP1} were replaced with Sir2$^{2-562}$ by P-MPGR[†] using pSir2-YIp5 (Shore et al., 1984) as template.
pCC4	*TRP1*	Sir3-Sir2$^{243-562}$	2, 3	Terminal Sir3^{3xHA} epitopes of pSIR3-HA^{TRP1} were replaced with Sir2$^{243-562}$ by P-MPGR.
pCC7*	*TRP1*	Sir2	4, 5, 6[‡]	SIR3$^{1-966}$ was removed from pCC1 by O-MPGR.
pCC8*	*TRP1*	Sir2$^{243-562}$	4, 5, 6 [‡]	Sir3-Sir2$^{2-242}$ portion of pCC1 was removed by O-MPGR[†].
pCC10	*TRP1*	Sir3-Hos3$^{2-549}$	7, 8	Terminal Sir3^{3xHA} epitopes of pSIR3-HA^{TRP1} were replaced with Hos3$^{2-549}$ by P-MPGR using genomic W303-1A DNA as template. A PCR error created a premature stop codon at position 550.
pCC11	*TRP1*	Sir3-Hos3$^{2-549AA}$	9, 12	Active site residues of Hos3$^{2-549}$ (H235, H236) in pCC10 were mutated to alanine by O-MPGR.
pCC18	*TRP1*	LexA-Sir2$^{243-562}$	13, 14	The Sir3-Sir2$^{2-242}$ portion of pCC4 was replaced with LexA by P-MPGR using pBTM116 as a template.
pCC21*	*TRP1*	Hos3$^{1-549}$	4, 9	The Sir3 portion of pCC10 was removed by O-MPGR.
pCC27	*TRP1*	LexA-Sir2$^{78-562}$	13, 29	Sir3-Sir2$^{2-77}$ portion of pCC1 was replaced with LexA by P-MPGR.
pCC29[#]	*TRP1*	LexA-Sir$^{78-252}$-Hos3$^{2-549}$-Sir2$^{522-562}$	25, 26	The Sir2$^{253-521}$ portion of pGLC117 was replaced with Hos3$^{2-549}$ by P-MPGR.
pCC30[#]	*TRP1*	LexA-Sir$^{78-252}$-Hos3$^{2-549AA}$-Sir2$^{522-562}$	25, 26	The Sir2$^{253-521}$ portion of pGLC117 was replaced with Hos3$^{2-549AA}$ by P-MPGR.
pCC31	*ADE2*	Sir3-Hos3$^{2-549}$	27, 28	The *TRP1* ORF of pCC10 was replaced with the *ADE2* ORF by P-MPGR using pM3938 (Voth et al., 2003) as template.
pCC32	*ADE2*	Sir2	27, 28	The *TRP1* ORF of pCC7 was replaced with the *ADE2* ORF by P-MPGR.
pCC33	*ADE2*	Sir3-Hos3$^{2-549AA}$	27, 28	The *TRP1* ORF of pCC11 was replaced with the *ADE2* ORF by P-MPGR.

[†] Primers (listed in table S3) were used in either P-MPGR (PCR-Mediated Plasmid Gap Repair) or O-MPGR (Oligonucleotide-Mediated Plasmid Gap Repair) to construct the respective clones.

***** The expression construct contains a leader peptide consisting of the last 12 amino acids of Sir3 (Sir3$^{967-978}$) inserted behind the initiating methionine codon.

[#] The expression construct is transcribed from the *ADH1* promoter.

[‡] Reaction contained three overlapping primers to make an extended polynucleotide product.

Table S3. Oligonucleotides for strain and plasmid construction, ChIP and RT-PCR.

#	Name	Sequence
1	*Sir3Sir2_1*	5’-taaattacgcattttcgatggatgaagaattcaaaaatatggactgcattACCATCCCACATATGAAATACG-3’
2	*Sir3Sir2_2*	5’-gcatgtgtacataggcatatttatggcggaagtgaaaatgaatgttggtggTTAGAGGGTTTTGGGATGTTC-3’
3	*Sir3HDAc_1*	5’-tacgcattttcgatggatgaagaattcaaaaatatggactgcattACTATTGATCATTTTATTCAAAAATTACAT-3’
4	*Sir3 splicing oligo*	5’-aaagttgttttgttctaacaattggattagctaaaatgTCGATGGATGAAGAATTCAAAAATATGGACTGCATT5-3’
5	*Sir3 splicing_1*	5’-ccctttcatcaccttccttacagaggtttaagAAAGTTGTTTTGTTCTAACAATTGG-3’
6	*Sir3 splicing_2*	5’-gtctttgatacggcgtatttcatatgtgggatggtAATGCAGTCCATATTTTTGAATTC-3’
7	*Sir3Hos3_1*	5’-taaattacgcattttcgatggatgaagaattcaaaaatatggactgcattTCTTCCAAGCATTCAGATCC-3’
8	*Sir3Hos3_2*	5’-gcatgtgtacataggcatatttatggcggaagtgaaaatgaatgttggtggTCACCATCTTCCACCACTTC-3’
9	*Hos3-r1900int*	5’-AGCTCATGGTCTGAATCTTC-3’
12	*Hos3HH235AA*	5’-acgcatgttgtcgtgcttgatttcgatctaGCCGCCggCgatgggacccaagacatttgctggaaacgtgcgggt-3’
13	*LexAdsir3chimera_F*	5’-agttgttttgttctaacaattggattagctaaaATGAAAGCGTTAACGGCC-3’
14	*LexAdsir3chimera_R*	5’-aatgcagtccatatttttgaattcttcatccatcgaCGGGAATTCCAGCCAGTC-3’
17	*HMR-E/5*	5’-CGCATTTCTTTTCGTCCACATTTGC-3’
18	*HMR-E/3*	5’-GATTAAGCTCATAACTTGGACGGG-3’
19	*A1/5*	5’-ATGGATGATATTTGTAGTATGGCG-3’
20	*A1/3*	5’-GGTGGTATATTTCTAACCTATTGTTAG-3’
21	*HMR-I/5*	5’-GAAGAGACTTATGATCAACATAATTTTGC-3’
22	*HMR-I/3*	5’-CATTTTCTTGTGCAAATTCCAA-3’
23	*ACT1/5*	5’-CTTCCACGTCCTCTTGCAT-3’
24	*ACT1/3*	5’-GCGTGAAAAATCTAAAAGCTGATG-3’
25	*sir2hos3_1*	5’-AATTTTTTCACTATTGATCATTTTATTCAAAAATTACATTCTTCCAAGCATTCAGATCC-3’
26	*Nsir2hos3tsir2c-2*	5’-CCATTTCTTATGCGGAATCGTCCAGCCACATTTTTGCTGTTTTTCCGTGCTTCTCGG-3’
27	*TRP1ORF_1*	5’-ATGTCTGTTATTAATTTCACAG-3’
28	*TRP1ORF_2*	5’-CTATTTCTTAGCATTTTTG-3’
29	*SIR2-int-nc-r*	5’-AGCGGTATGTAATTTTTG-3’
30	*YFR057W-f*	5’-CTAGTGTCTATAGTAAGTGCTCGG-3’
31	*YFR057W-r*	5’-GGTATATTGCCACGCAAAGAAAGG-3’
32	*KCC4-f*	5'-TTGGTGAAACACTGGGCTTT-3'
33	*KCC4-r*	5'-GGCGTATTCCAGGATAAGGTA-3'

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