Table S1. Plasmids

Name	Marker/Vector*/Promoter	Protein	Primers§	Construction†
pJR368	*URA3/CEN/SIR4p*	Sir4	-	From J. Rine (UC Berkeley)
pPCM14tetO	*(URA3,ADE2,LEU2/ CEN/NA)*	NA	-	From P. Megee (UC Denver)
pGAD-C1	*LEU2/2μ/ADH1p*	$Gal4_{AD}$	-	(1)
pBTM116	*TRP1/2μ/ADH1p*	lexA	-	“”
pBTM116H	*HIS3/2μ/ADH1p*	lexA	-	(2)
pCSW22	*HIS3/2μ/ADH1p*	lexA-$Sir2^{78-562}$		“”
pCSW23	*HIS3/2μ/ADH1p*	lexA-$Sir2^{243-562}$-H364Y	-	“”
pCSW55	*TRP1/2μ/ADH1p*	lexA-$Sir2^{243-562}$-H364Y	-	“”
pYFC1	*HIS3/2μ/ADH1p*	lexA-$Sir2^{499-562}$	-	“”
pYFC6	*HIS3/2μ/ADH1p*	lexA-$Hst1^{440-503}$	-	“”
pYFC10	*HIS3/2μ/ADH1p*	lexA-$Sir2^{499-562}$-AKAA	3/4	The *AKAA* mutation was placed in pCSW23 by P-MPGR using pYFC1 as template.
pYFC12	*HIS3/2μ/NA*	$Sir2^{364-562}$-AKAA	5/6, 7/8	A Sir2 fragment with *AKAA* mutation was cloned into pRS423 by P-MPGR.
pYFC13	*HIS3/Int/NA*	$Sir2^{364-562}$-AKAA		A *2μ* fragment was removed from pYFC12 by *Afe*I digestion and religation.
pYFC20	*HIS3/2μ/ADH1p*	lexA-$Sir2^{243-562}$-H364Y-AKAA	20/6, 7/21	The *AKAA* mutation was placed in pCSW23 by P-MPGR using pCSW23 as template.
pYFC21	*TRP1/2μ/ADH1p*	lexA-$Sir2^{243-562}$-H364Y-AKAA		Marker swap of pYFC20 by P-MPGR.
pYFC29	*HIS3/Int/NA*	$Sir2^{364-562}$-AKAK	9/10	The *sir2* mutation was placed in

				*Pst*I-cut pYFC12 by p-MPGR. A *2μ* fragment was removed by *Afe*I digestion and religation.
pYFC30	*HIS3/Int/NA*	$Sir2^{364\text{-}562}$-AKDK	11/12	""
pYFC31	*HIS3/Int/NA*	$Sir2^{364\text{-}562}$-EKAK	13/14	""
pT2-4-14	*LEU2/2μ/ADH1p*	$GAL4_{AD}$-$ESC8^{640\text{-}714}$	-	Isolated from library (1).

* high-copy plamid (*2μ*), low-copy plasmid (CEN) or integration vector (Int).
§ Primers used for P-MPGR are listed in Table S2.
† P-MPGR (PCR-Mediated Plasmid Gap Repair)

Table S2. PCR primers

#	Name	Sequence (5'-3')
3	lexA Cter	CTGTTGCCAGAAAATAGCGA
4	R primer for AKAA mutant	GACATACACGCCtgcagcCTTagcTTGGCATTT
5	Forward Sir2 at *Nco*I	gtaatacgactcactatagggcgaattgggtaccgggcATGGCTCTTTTGCTACTGCCAC
6	Reverse Sir2 at AKAA	ATCTGATGTAACGACATACACGCCtgcagcCTTagcTTGGCATTTAAAGTTCTTGTTCTTC
7	Forward Sir2 before AKAA	gctgcaGGCGTGTATGTCGTTACATCAGAT
8	Reverse Sir2 after orf	caattaaccctcactaaagggaacaaaagctggagctcTTTGAATGTATGGATGCGTTAAATG
9	AKAK-F	GAACGATTTGAAGAACAAGAACTTTAAATGCCAAgctAAGgctAAGGGCGTGTATGTCGTTACATCAGATGAACATCCCA
10	AKAK-R	TGGGATGTTCATCTGATGTAACGACATACACGCCCTTagcCTTagcTTGGCATTTAAAGTTCTTGTTCTTCAAATCGTTC
11	AKDK-F	GAACGATTTGAAGAACAAGAACTTTAAATGCCAAgctAAGGATAAGGGCGTGTATGTCGTTACATCAGATGAACATCCCA
12	AKDK-R	TGGGATGTTCATCTGATGTAACGACATACACGCCCTTATCCTTagcTTGGCATTTAAAGTTCTTGTTCTTCAAATCGTTC
13	EKAK-F	GAACGATTTGAAGAACAAGAACTTTAAATGCCAAGAGAAGgctAAGGGCGTGTATGTCGTTACATCAGATGAACATCCCA
14	EKAK-R	TGGGATGTTCATCTGATGTAACGACATACACGCCCTTagcCTTCTCTTGGCATTTAAAGTTCTTGTTCTTCAAATCGTTC
20	Sir2-63801	Gccaatatggttttacctccag
21	ADH1-t-R	TAATAAAAATCATAAATC
22	a1-1 (rtPCR)	cattctaggtactgagattgatgaaa
23	a1-2 (rtPCR)	cgtgcttggggtgatattgat
24	KCC4-f	TTGGTGAAACACTGGGCTTT
25	KCC4-r	GGCGTATTCCAGGATAAGGTA
26	a2-3'	GCGGATGGGTTGGTATTTAA
27	a2-5'	ACAAATCTAGAAATTACCAGAGCTATC
28	549.7 New S	TGAAGATGACATTGCTCCTTT
29	549.7 New AS	TGGATAATGGATCTGAAACCG
30	534 S	ACAAGCATCATTCATAGCCT
31	534 AS	ATCGTGGCTAGGACATTTTG

Table S3. Yeast strains

Name	Genotype	Reference
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(3)
YDS631	W303-1A *MATα adh4::URA3::*$C_{1\text{-}3}$*A*	(4)
L40	*MAT***a** *his3-Δ200 trp1-901 leu2-3,112 ade2 lys2-801am URA3::(8xlexA*^{op}*)-lacZ LYS2::(4xlexA*^{op}*)-HIS3*	(5)
MC52	W303-1A *MATα URA3::ADE2-TelVIIL SUM1-1*	(6)
PMY127	W303-1A *his3-11,15::(GAL1p-RecR::HIS3)*$_n$ *LEU2::tetR-GFP::leu2-3,112 bar1*	(7)
DMY2800	W303-1A *IGS2::TRP1p-URA3::LEU2*	(8)
DMY2804	W303-1A *IGS1::TRP1p-URA3::LEU2*	“”
DMY2831	DMY2800 *sir2Δ::kanMX6*	“”
DMY2835	DMY2804 *sir2Δ::kanMX6*	“”
GCY310	GCY317 *esc8Δ::kanMX*	(9)
GCY317	W303-1A *hmrΔA::ADE2::HMRI*	“”
GA-2050	W303-1A *MATα hmrΔEB-4lex*^{op}*-TRP1::HMRI*	(10)
THC74	W303-1A *URA3 HML::URA3p–ADE2*	(11)
CSW10	W303-1A *RS::HMRE-***a***2***a***1-HMRI-TRP1-256xlac*^{op}*::RS (LEU2::GAL1p-R)*$_2$*::leu2-3,112 ADE2::HIS3p-lacGFP::ade2-1*	(12)
CRC83	CSW10 *MATα scc1-73*	“”
CSW42	W303-1A *RS::6xlex*^{op}*ssEB-***a***2***a***1-256xlac*^{op}*-TRP1-hmrΔI::RS (LEU2::GAL1p-R)*$_2$*::leu2-3,112 ADE2::HIS3p-lacGFP::ade2-1 bar1Δ::hisG sir2Δ::klURA3*	(2)
CSW84	CSW10 *sir2Δ::hphMX*	“”
CSW98	GA-2050 *sir2Δ::hphMX*	
CSW116	W303-1A *MATα MCD1-TAP::natMX*	“”
CSW157	GA-2050 *sir4Δ::kanMX*	
CCC1	YDS631 *sir2Δ::kanMX*	(13)

CCC3	THC74 *sir2Δ::kanMX*	“”
YFC1	CSW10 *sir2-AKAA::HIS3*	This study
YFC3	DMY2800 *sir2-AKAA::HIS3*	“”
YFC7	CSW116 *sir2-AKAA::HIS3*	“”
YFC8	CSW84 *MAT***a** *SUM1-1 URA3::ADE2-TelVIIL*	“”
YFC9	CSW116 *sir2Δ::hphMX*	“”
YFC12	YDS631 *sir2-AKAA::HIS3*	“”
YFC13	DMY2804 *sir2-AKAA::HIS3*	“”
YFC18	CSW10 *esc8Δ::kanMX*	“”
YFC21	CSW42 *esc8Δ::kanMX*	“”
YFC24	THC74 *sir2-AKAA::HIS3*	“”
YFC35	L40 *sir2Δ::kanMX*	“”
YFC43	CSW84 *esc8Δ::kanMX*	“”
YFC44	GCY317 *sir2-AKAA::HIS3*	“”
YFC45	CSW42 *ioc3Δ::kanMX*	“”
YFC95	CSW116 *esc8Δ::kanMX*	“”
YFC96	PMY127 *esc8Δ::kanMX*	“”
YFC97	YFC8 *hst1Δ::kanMX*	“”
YFC101	CSW116 *MAT***a** *SUM1-1 sir2Δ::hphMX*	“”
YFC103	YFC101 *hst1Δ::kanMX*	“”
YFC108	PMY127 *mcm21Δ::kanMX*	“”
YFC109	W303-1A *RS::HMRE-***a**2**a***1-HMRI-TRP1-256xlac^{op}::RS (LEU2::GAL1p-R)$_2$::leu2-3,112 ADE2::HIS3p-lacGFP::ade2-1 SUM1-1 scc1-73 sir2Δ::hphMX*	“”
YFC116	GCY317 *sir2Δ::hphMX*	“”
YFC117	GCY310 *sir2Δ::hphMX*	“”

YFC128	YFC45 *esc8Δ::hphMX*	“”
YFC143	CSW42 *rfm1Δ::kanMX*	“”
YFC135	CSW42 *isw1Δ::kanMX*	“”
YFC145	CSW42 *ioc4Δ::kanMX*	“”
YFC130	CSW10 *ioc3Δ::kanMX*	“”
YFC133	CSW10 *isw1Δ::kanMX*	“”
YFC144	CSW10 *ioc4Δ::kanMX*	“”
YFC137	CSW10 *sir2-AKAK::HIS3*	“”
YFC138	CSW10 *sir2-AKDK::HIS3*	“”
YFC139	CSW10 *sir2-EKAK::HIS3*	“”
YFC140	GCY317 *sir2-AKAK::HIS3*	“”
YFC141	GCY317 *sir2-AKDK::HIS3*	“”
YFC142	GCY317 *sir2-EKAK::HIS3*	“”
YFC150	CSW10 *matΔ::natMX*	“”
YFC151	CSW84 *matΔ::natMX*	“”
YFC152	YFC1 *matΔ::natMX*	“”
YFC153	YFC18 *matΔ::natMX*	“”
YFC154	CSW10 *MCD1-TAP::natMX*	“”
YFC155	CSW84 *MCD1-TAP::natMX*	“”
YFC156	YFC1 *MCD1-TAP::natMX*	“”
YFC157	YFC18 *MCD1-TAP::natMX*	“”

All strains were derived from W303-1A except L40 and YFC35.

References for Tables

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Figure S1
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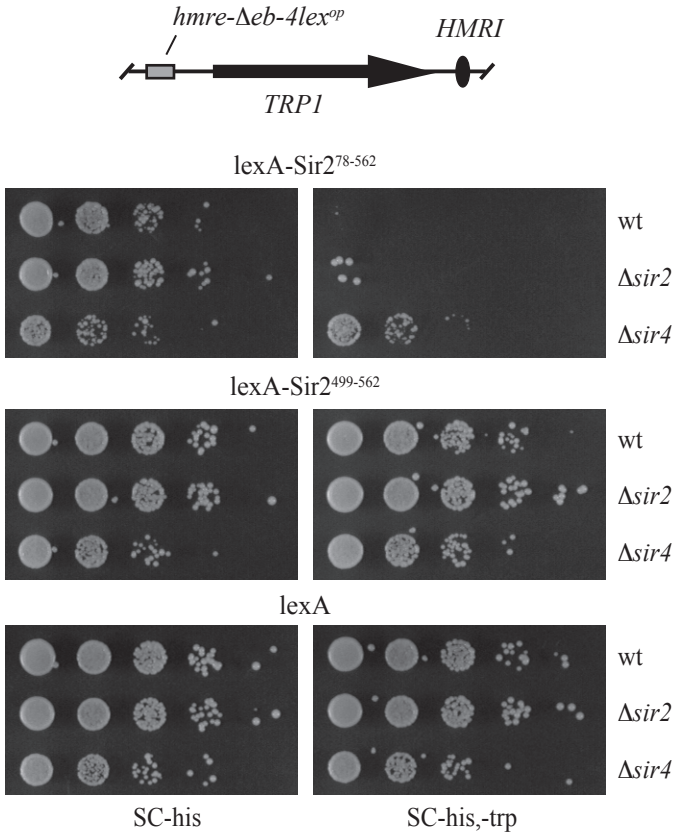

Figure S1 – The cohesion proficient domain of Sir2 (amino acids 499-562) is not sufficient for targeted silencing. Strains GA-2050 (wt), CSW98 (*sir2Δ*) and CSW157 (*sir4Δ*) expressing lexA-Sir2$^{499\text{-}562}$ (pYFC1), lexA-Sir2$^{78–562}$ (pCSW22) or lexA alone (pBTM116H) were spotted on SC-trp,-his plates to measure silencing and on SC-his as a loading control. lexA-Sir2$^{499\text{-}562}$ does not nucleate silent chromatin but lexA-Sir2$^{78\text{-}562}$ does. Similar results were obtained for lexA-Sir2$^{78\text{-}562}$ when the chimera was tethered to a locus that lacks the *HMR I* silencer (Chia-Ching Chou, PhD Thesis, 2008).

Figure S2
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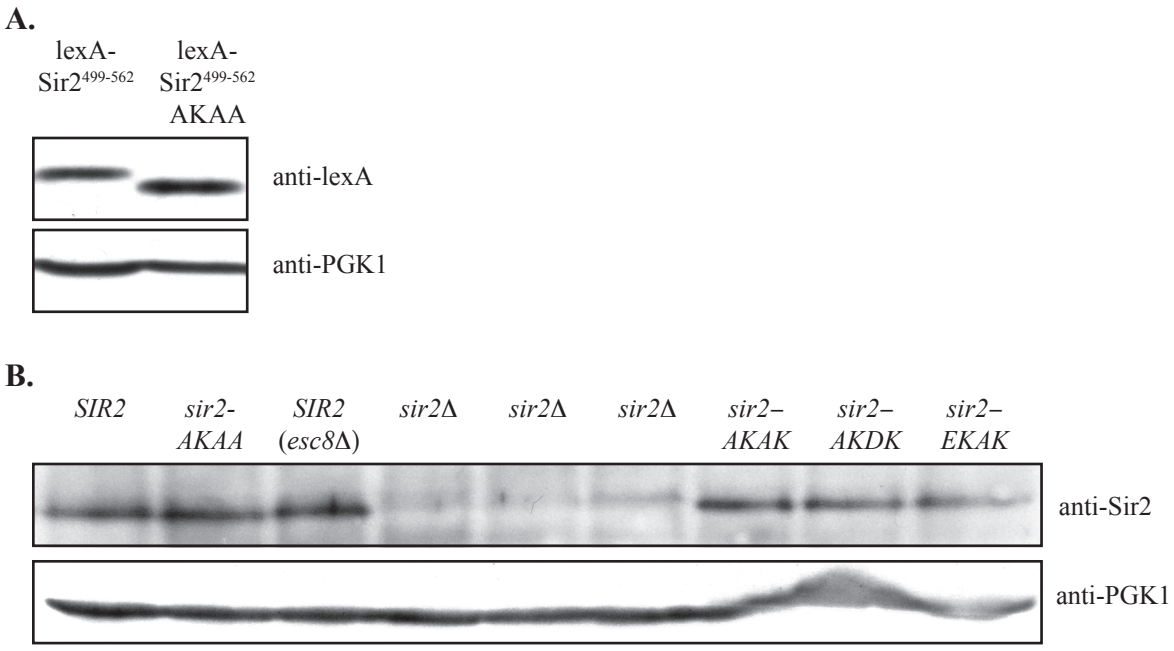

Figure S2 – Sir2 protein levels in the strains used in this study. A) Levels of lexA-Sir2 chimeras. Immunoblotting was performed with strain CSW42 (*sir2Δ*) expressing either lexA-Sir2$^{499\text{-}562}$ (pYFC1) or lexA-Sir2$^{499\text{-}562}$-AKAA (pYFC10). B) Levels of full length Sir2. Strains CSW10 (wt), CSW84 (*sir2Δ*), YFC1 (*sir2-AKAA*), YFC18 (*esc8Δ*), YFC137 (*sir2-AKAK*), YFC138 (*sir2-AKDK*), and YFC139 (*sir2-EKAK*) were used. Sir2 levels were not affected by mutation of the EKDK motif or deletion of *ESC8*.

Figure S3
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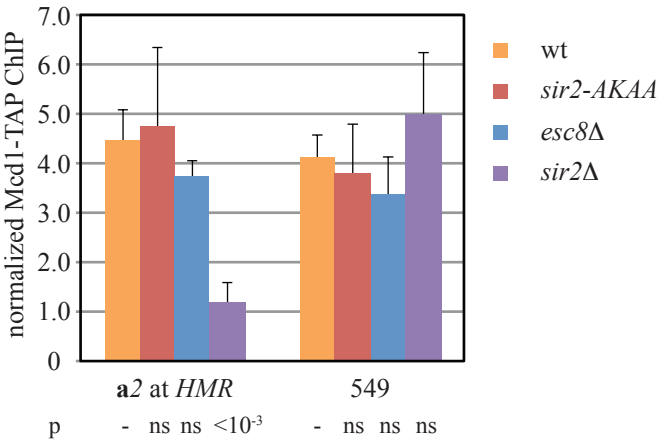

Figure S3 – Cohesin binding at *HMR* in DNA circles. ChIP of Mcd1-TAP was performed with strains YFC154 (wt), YFC155 (*sir2Δ*), YFC156 (*sir2-AKAA*) and YFC157 (*esc8Δ*) two hours after the addition of galactose to form DNA circles. p values for student's t-test are reported relative to the wild-type strain.

Figure S4
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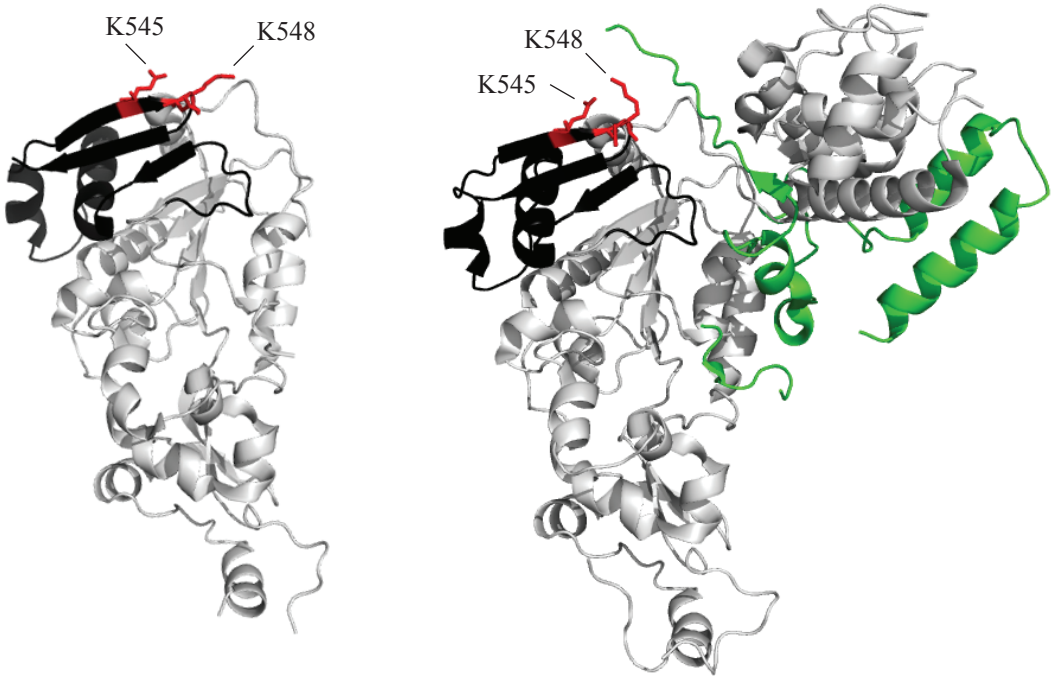

Figure S4 – Positions of the EKDK motif within crystal structures of Sir2 alone and Sir2 with Sir4. Mutated residues are shown in red; the cohesion proficient domain of Sir2 is shown in black and the remainder of crystalized Sir2 is shown in grey. The Sir4 fragment is shown in green. Structures are based on coordinates from PDB accession numbers 2HJH and 4AIO).

Figure S5
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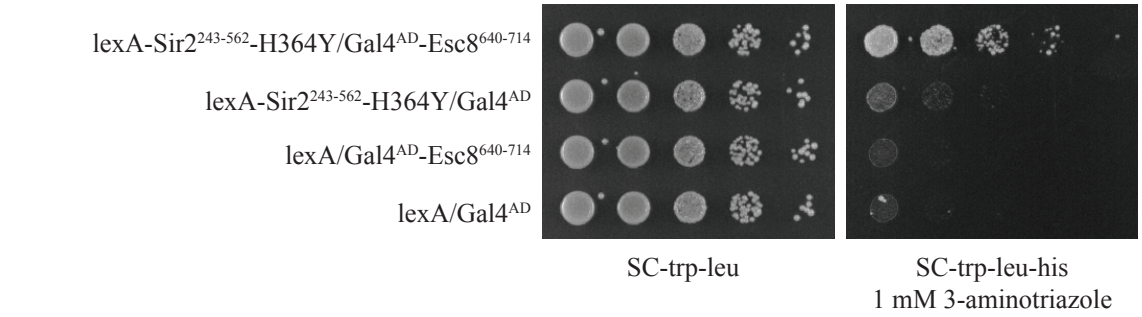
A.



B.

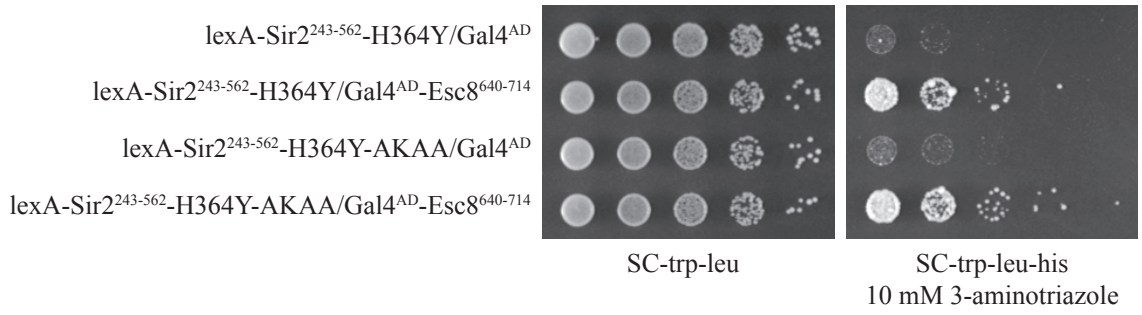


Figure S5 – Two-hybrid interaction between Esc8 and Sir2. Candidate interacting factors were isolated according to protocol in Materials and Methods. **A)** Strain YFC35 (*sir2Δ*) expressing pairwise combinations of lexA-Sir2$^{243\text{-}562}$-H364Y (pCSW55), GAL4$_{AD}$-Esc8$^{640\text{-}714}$ (pT2-4-14), lexA alone (pBTM116) and GAL$_{AD}$ alone (pGAD-C1) were spotted on SC-trp-leu as a loading control and SC-trp-leu-his plus 1 mM 3-AT to score interactions. **B)** Strain YFC35 (*sir2Δ*) expressing pairwise combinations of lexA-Sir2$^{243\text{-}562}$-H364Y (pCSW55), lexA-Sir2$^{243\text{-}562}$-H364Y-AKAA (pYFC21), GAL4$_{AD}$-Esc8$^{640\text{-}714}$ (pT2-4-14) and GAL$_{AD}$ alone (pGAD-C1) were spotted on SC-trp-leu as a loading control and SC-trp-leu-his plus 10 mM 3-AT to score interactions. The *AKAA* mutation does not disrupt the association of Esc8 with Sir2.

Figure S6
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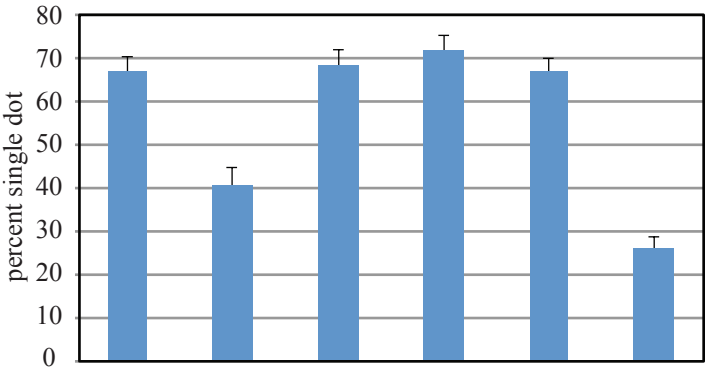

	lexA-Sir2$^{78\text{-}562}$	lexA-Sir2$^{78\text{-}562}$ *isw1*Δ	lexA-Sir2$^{78\text{-}562}$ *esc8*Δ	lexA-Sir2$^{78\text{-}562}$ *esc8*Δ *ioc3*Δ	lexA-Sir2$^{78\text{-}562}$ *ioc4*Δ	lexA alone
N	215	147	177	185	219	346
p	-	$<10^{-07}$	ns	ns	ns	$<10^{-21}$

Figure S6 – Cohesion of silent chromatin nucleated by lexA-Sir2$^{78\text{-}562}$ requires Isw1 but not Esc8 or the Ioc proteins. Cohesion of DNA circles bearing lexA sites was evaluated in strains CSW42 (*sir2Δ*), YFC135 (*sir2Δ isw1*Δ), YFC21 (*sir2Δ esc8Δ*), YFC128 (*sir2Δ ioc3Δ esc8Δ*) and YFC145 (*sir2Δ ioc4Δ*) expressing lexA-Sir2$^{78\text{-}562}$ (pCSW22). Strain CSW42 expressing lexA alone (pBTM116H) was included as a negative control. Cohesion mediated by tethered Sir2 does not require *ESC8*, *IOC3 or IOC4* even though lexA-Sir2$^{78\text{-}562}$ nucleates silent chromatin when targeted to DNA (See figure S1).