

Appendix

Sir3 Heterochromatin Protein Promotes NHEJ by Direct Inhibition of Sae2

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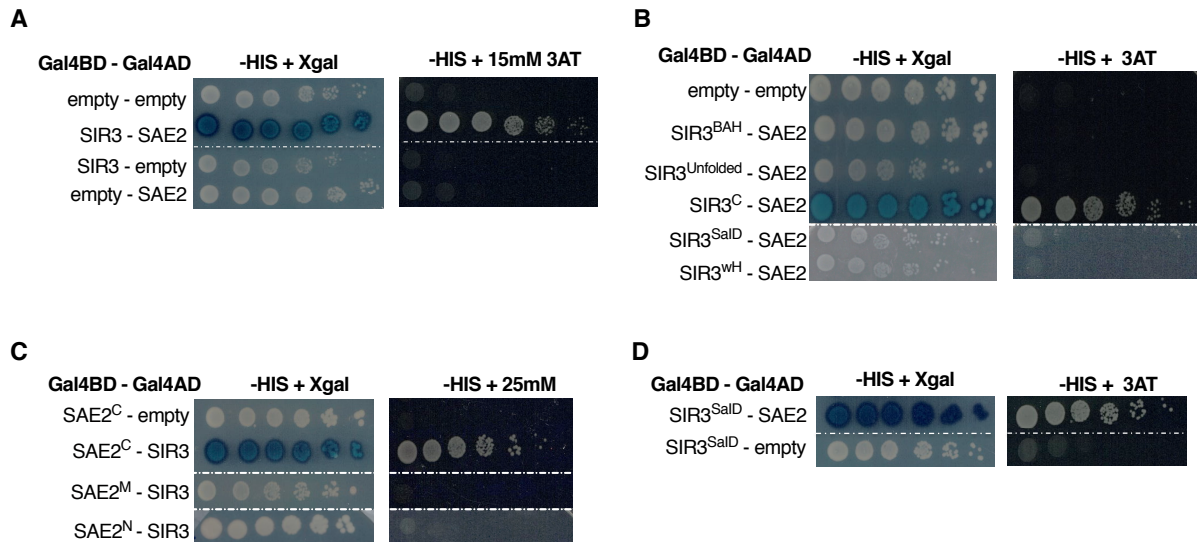
Appendix Figure S1

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Appendix Figure S3

Appendix Table S1

Appendix Table S2



Yeast two-hybrid analysis of Sir3-Sae2 interaction

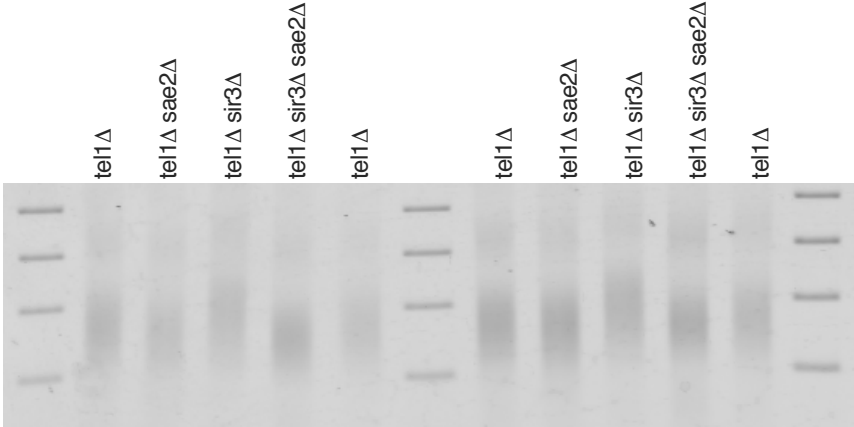
Y2H screen analysis was performed in WT cells on -His + 3AT media and X gal coloration.

A. Representative image of two hybrid assays testing the interaction of the full length WT Sae2 and Sir3 proteins.

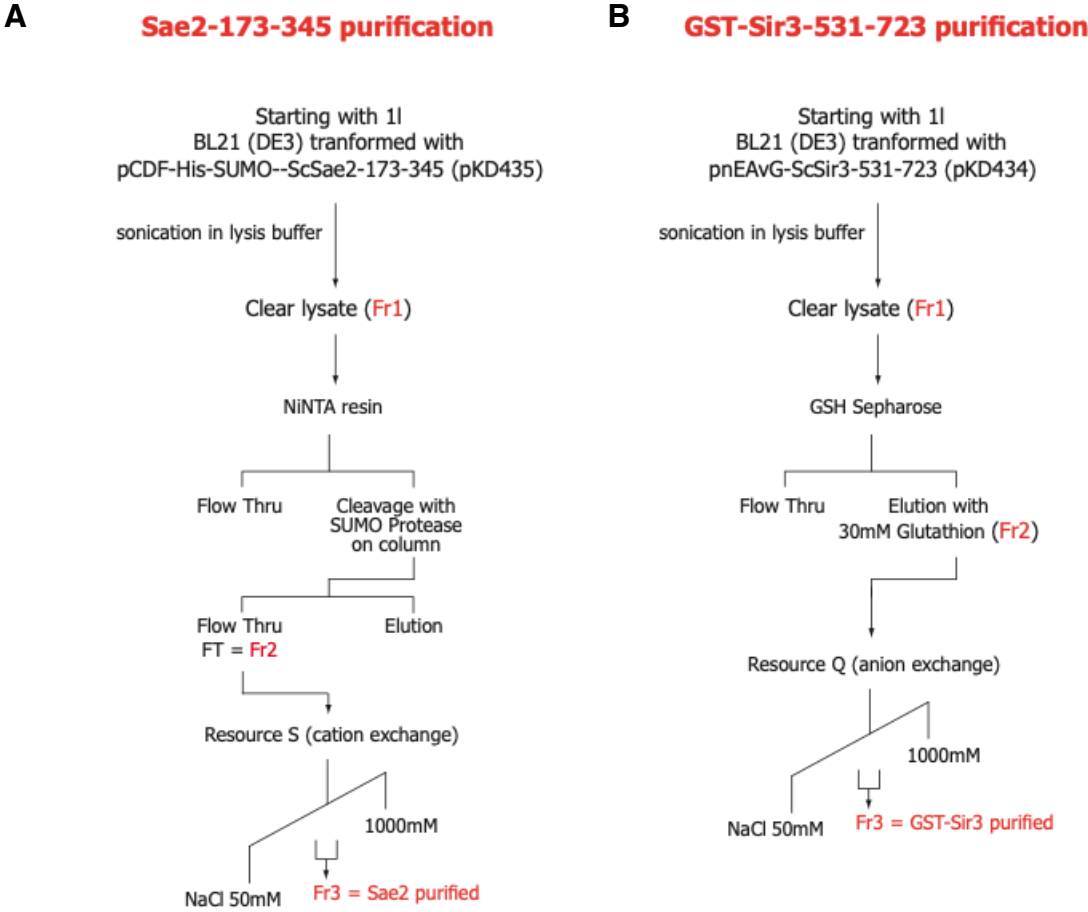
B. Representative image of two hybrid assays testing the interaction of full length Sae2 with Sir3 fragments. SIR3^{BAH}= aa 1-216, SIR3^{unfolded}= aa 217-530 SIR3^C= aa 531-848, SIR3^{WH}= aa 849-978 and SIR3^{Sae2}= 531-723.

C. Representative image of two hybrid assays testing the interaction of full length Sir3 with Sae2 fragments. SAE2^C= aa 1-72 , SAE2^M= aa 73-172 , SAE2^N= aa 173-345.

D. Representative image of two hybrid assays testing the interaction of Sir3^{Sae2} and Sae2^C with full length Sae2 and Sir3 respectively.



Representative electrophoresis of telomere-PCR products of tel1Δ strains combined with the indicated null mutations. Telomere length was assessed by PCR after end-labeling with terminal transferase (Teixeira et al 2004).



Scheme of the purification procedure for Sae2 (A) and Sir3 (B) fragments

Appendix Table S1: *S. cerevisiae* strains used in this paper:

Strain		Genotype	Source or reference	figure
yKD513	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx	This study	1B
yKD788	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx dnl4Δ::HIS3Mx	Batté et al. 2107	1B
yKD790	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx sir3::Nat-GPDp-SIR3	This study	1B, 2C
yKD800	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx dnl4Δ::HIS3Mx sir3::Nat-GPDp-SIR3	Batté et al. 2107	1B
yKD1891	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx sir3::Nat-GPDp-SIR3 sir4Δ::HIS3Mx	This study	1B
yKD1472	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx sae2Δ::HIS3Mx	This study	1B
yKD1561	(1)	TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx sae2Δ::HIS3Mx sir3::Nat-GPDp-SIR3	This study	1B
yKD516	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx	This study	1C, 1E, 1H, 5A, 5B, 6A, 6B
yKD789	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx dnl4Δ::HIS3Mx	Batté et al. 2107	1C, 6B
yKD706	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx sir3::Nat-GPDp-SIR3	This study	1C, 1E, 1H, 5B, 6B
yKD802	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx dnl4Δ::HIS3Mx sir3::Nat-GPDp-SIR3	Batté et al. 2107	1C, 6B
yKD1562	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx dnl4Δ::HIS3Mx sir3::Nat-GPDp-SIR3 sae2Δ::HIS3Mx	This study	1C, 1E, 1H, 6B
yKD1474	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx sae2Δ::HIS3Mx	This study	1C, 1E, 1H, 5A, 5B, 6A, 6B
yKD1656	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx mre11-H125N	This study	1C
yKD2274	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx mre11-H125N sae2Δ::Nat	This study	1C
yKD1880		MATa RAD5 ADE2 hmlΔ::HPH trp1:: Gal1p-ISceI-TRP1 lys2::lox-ura3-ISceI ura3-1Δ::KanMx Mre11-YFP	This study	1F, 1G, 7B
yKD1881		MATa RAD5 ADE2 hmlΔ::HPH trp1:: Gal1p-ISceI-TRP1 lys2::lox-ura3-ISceI ura3-1Δ::KanMx Mre11-YFP sir3::Nat-GPDp-SIR3	This study	1F, 1G, 7B
yKD1841		MATa RAD5 ADE2 hmlΔ::HPH trp1::GAL1p-ISceI-TRP1 lys2::lox-ura3-ISceI ura3-1Δ::KanMx Mre11-YFP sae2Δ::HIS3Mx	This study	1G
yKD1843		MATa RAD5 ADE2 hmlΔ::HPH trp1::GAL1p-ISceI-TRP1 lys2::lox-ura3-ISceI ura3-1Δ::KanMx Mre11-YFP sae2Δ::HIS3Mx sir3::Nat-GPDp-Sir3	This study	1G
yKD1620	(1)	lys2::lox-ura3-ISceI ura3-1Δ::KanMx sir3::Nat-ADH1p-SIR3	This study	1H
yKD1845		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-ISceI-TRP1 TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::SIR3-mCherry-HPH	This study	2A
yKD1864		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-ISceI-TRP1 TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-SIR3-mCherry-HPH	This study	2A, 5C
yKD1664		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1:: GAL1p-SceI-TRP1 TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMX	This study	2B, 2C, 2D, 5E, 6F
yKD1680		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-ISceI-TRP1 TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx SAE2-GFP-spHIS5	This study	2B, 2D, 5E, 7A
yKD1712		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-ISceI-TRP1 TELVI-R::lox-ura3-ISceI ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-SIR3	This study	2B, 2C, 5E, 7A

yKD1778		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-IScel-TRP1 TELVI-R::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3Δ::Nat	This study	2B, 2D, 5E
Y190		MATa his3-Δ200 ade2-101 trp1-901 leu2-3,112 cyh2 gal4Δ gal80Δ ura3-52::URA3::GAL1 _{UAS} -GAL1 _{TATA} -lacZ lys2-801::LYS2::GAL4 _{UAS} -HIS3 _{TATA} -HIS3	Harper et al 1993	3B,3C, 3D, 4C, 6E
yKD1882		Y190 sir4Δ::Nat	This study	3D
yKD1991		his3-Δ200 ade2-101 trp1-901 leu2-3,112 cyh2 gal4Δ gal80Δ URA3::lexAop-lacZ LYS2::GAL1 _{UAS} -HIS3 _{TATA} -HIS3	This study	4B
yKD2157	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3 ^{SaiD}	This study	5A
yKD2158	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3 ^{SaiD} sae2Δ::HIS3Mx	This study	5A
yKD2161	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3 ^{SaiD-T557I}	This study	5A
yKD2162	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3 ^{SaiD-T557I} sae2Δ::HIS3Mx	This study	5A
yKD2192	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3-T557I	This study	5B
yKD2194	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-GPDp-sir3-T557I sae2Δ::HIS3Mx	This study	5B
yKD2287		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-IScel-TRP1 TELVI-R::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-sir3-T557I-mCherry-HPH	This study	5C
yKD1932	(2)	adh4::URA3-TEL7L	This study	5D
yKD1934	(2)	adh4::URA3-TEL7L sir4Δ::Nat	This study	5D
yKD2181	(2)	adh4::URA3-TEL7L sir3::Nat-GPDp-SIR3	This study	5D
yKD2254	(2)	adh4::URA3-TEL7L sir3::Nat-GPDp-sir3-T557I	This study	5D
yKD2242	(2)	adh4::URA3-TEL7L sir3-T557I	This study	5D
yKD2286		MATalpha RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p-IScel-TRP1 TELVI-R::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-sir3-T557I	This study	5E
yKD1467	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3Δ::HIS3MX	This study	6A, 6B
yKD1892	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir4Δ::HIS3Mx	This study	6A
yKD1936	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3Δ::Nat sir4Δ::HIS3Mx	This study	6A
yKD1969	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir4Δ::HIS3Mx sae2Δ::Nat	This study	6A
yKD2265	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3-T557I	This study	6A
yKD2278	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3-T557I sir4Δ::Nat	This study	6A
yKD2038	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx his3::HIS3-TEFp	This study	6C
yKD2039	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx his3::HIS3-TEFp-SIR4	This study	6C
yKD2054	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-ADH1p-SIR3 his3::HIS3-TEFp	This study	6C
yKD2055	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-ADH1p-SIR3 his3::HIS3-TEFp-SIR4	This study	6C
yKD2042	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-ADH1p-SIR3 sae2Δ::Nat his3::HIS3-TEFp	This study	6C
yKD2043	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sir3::Nat-ADH1p-SIR3 sae2Δ::Nat his3::HIS3-TEFp-SIR4	This study	6C
yKD2133	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5	This study	7A, 7C, 7D
yKD2138	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3Δ::Nat	This study	7C, 7D
yKD2139	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-SIR3	This study	7A, 7C, 7D
yKD2141	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx SAE2-GFP-spHIS5 sir3::Nat-GPDp-SIR3 ^{SaiD}	This study	7A, 7C, 7D

yKD2334		MATa RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p- IScel-TRP1 TELVI-R::lox-ura3-IScel ura3-1Δ::KanMx SAE2- GFP-spHIS5 sir3::Nat-GPDp-SIR3 his3::HIS3-TEFp	This study	6D, 6F
yKD2335		MATa RAD5 ADE2 hmlΔ::oripRS hmrΔ::ampR trp1::GAL1p- IScel-TRP1 TELVI-R::lox-ura3-IScel ura3-1Δ::KanMx SAE2- GFP-spHIS5 sir3::Nat-GPDp-SIR3 his3::HIS3-TEFp-SIR4	This study	6D, 6F
yKD2355	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sae2-S267E-4ala-YFP	This study	7E, 7F
yKD2356	(1)	lys2::lox-ura3-IScel ura3-1Δ::KanMx sae2-S267E-4ala-YFP sir3::Nat-GPDp-SIR3	This study	7E, 7F

(1) All those strains are W303 *MATa RAD5+ ade2-1::ADE2 rap1::GFP-RAP1-LEU2 hml::HPH trp1::GAL1p-IScel-TRP1*

(2) All those strains are W303 *Mata RAD5 ADE2 leu2-3,112 his3-11,15 trp1-1 ura3-1*

Appendix Table S2: **Primers used in this paper:**

Gene	Primer Name	Primer Sequence
OGG1_F	pr776	CAATGGTGTAGGCCCCAAAG
OGG1_R	pr777	ACGATGCCATCCATGTGAAGT
TEL6R_+0.2kb_F	pr750	TGAGGCCATTTCCGTGTGTA
TEL6R_+0.2kb_R	pr751	CCCAGTCCTCATTTCATCAA
TEL6R_+0.9kb_F	pr752	TGATGAATTACAAGGGAACAATGAG
TEL6R_+0.9kb_R	pr753	CATCAAACAAGTAGGAATGCGAAA
LYS2_0.9kb_F	pr764	TGATTTACCATTGGGCACAATTT
LYS2_0.9kb_R	pr765	AATTTCCGCGGCAAAGG
IScelcs_F	pr768	GGAGTTAGTTGAAGCATTAGGTCCC
IScelcs_R	pr769	GCGGCTTAACTGTGCCCTC
LYS2_0.2kb_F	pr760	GCTCAAAGAGACATGGGTGGA
LYS2_0.2kb_R	pr761	TGTCATCTAAACCCACACCGG