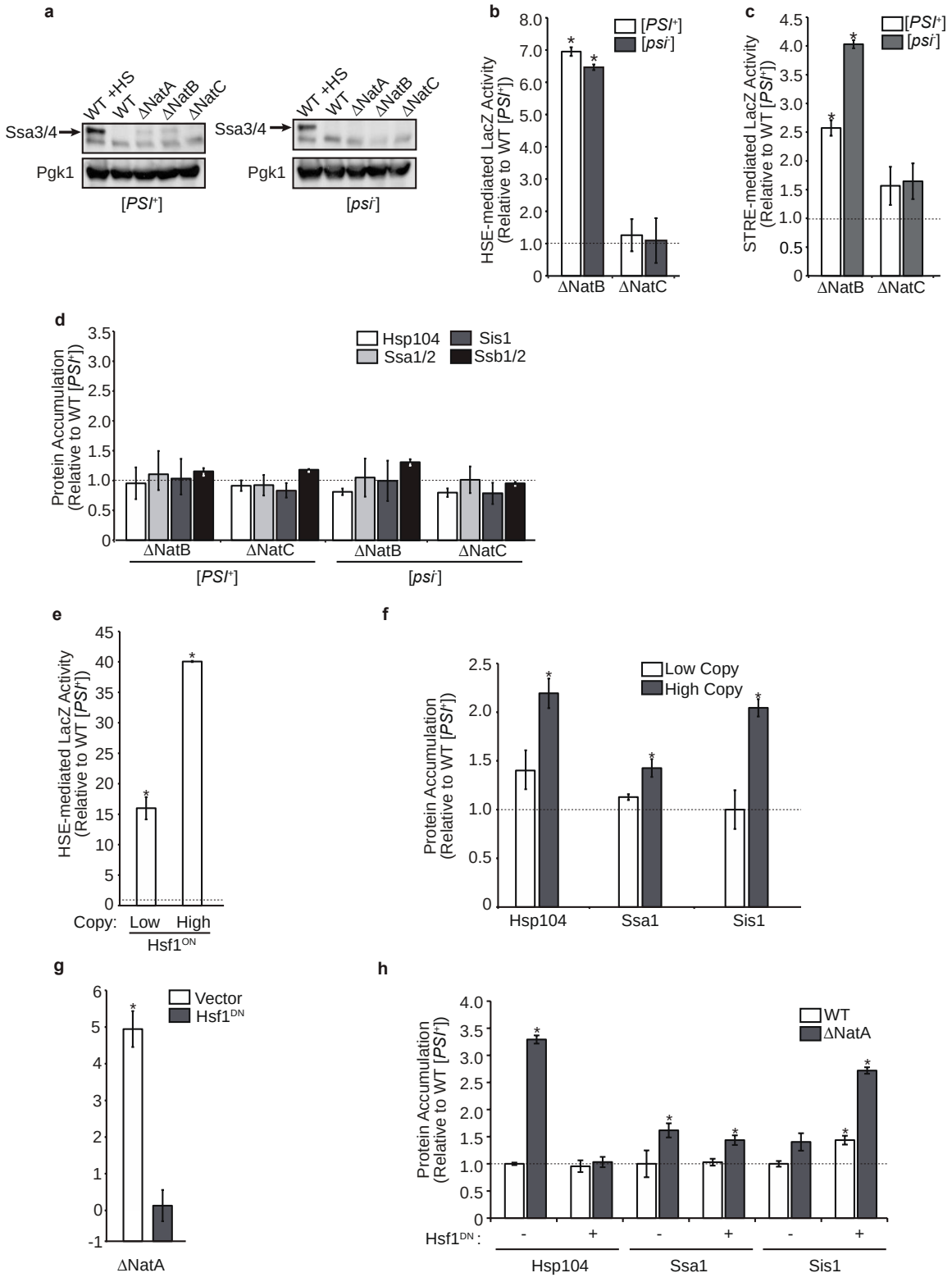
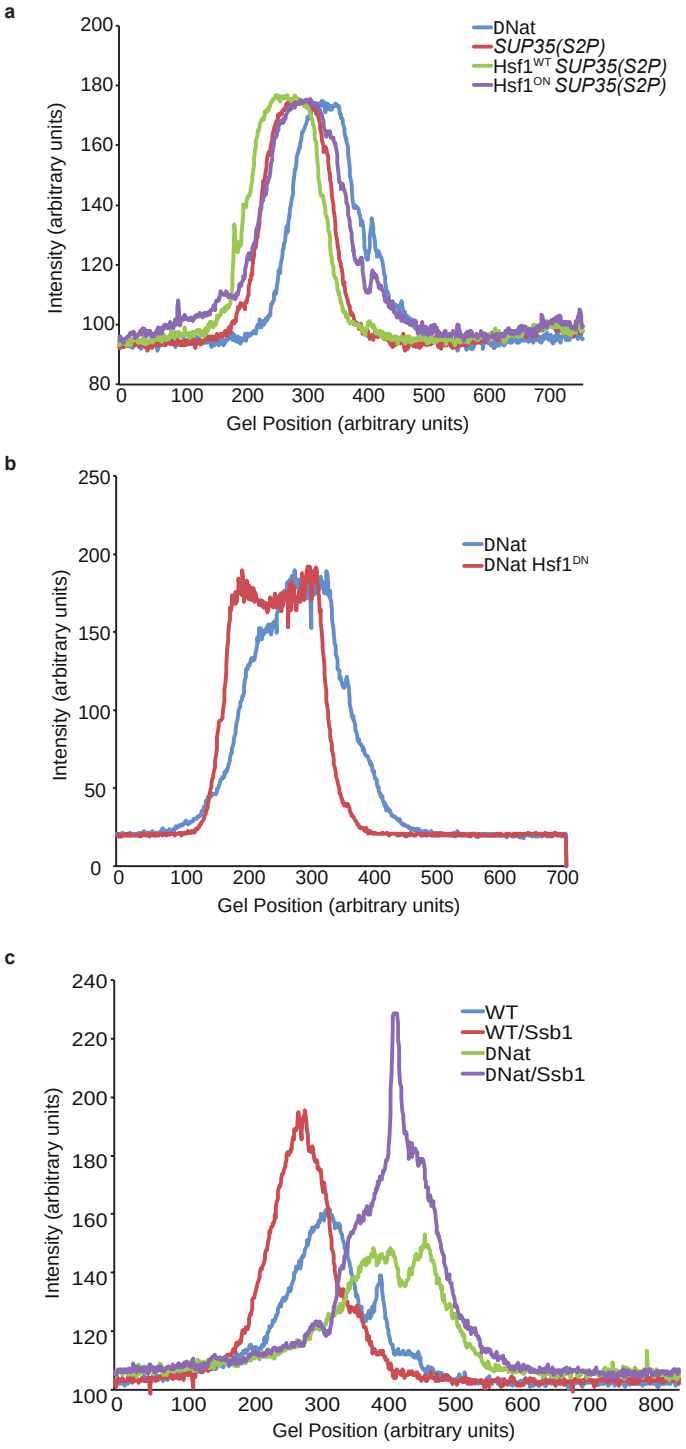
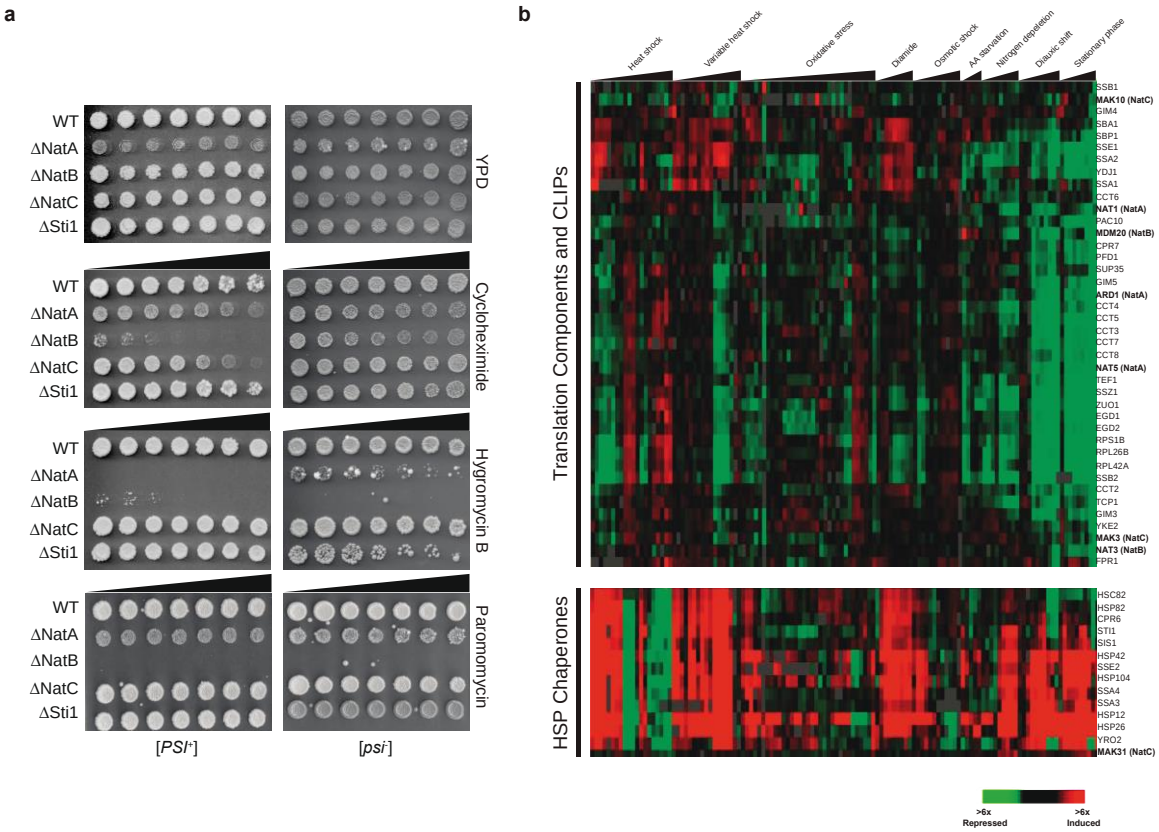

a
WT +HS
WT
ΔNatA
ΔNatB
ΔNatC
Ssa3/4
Pgk1
[PSI+]
[psi-]
b
[PSI+]
[psi-]
HSE-mediated LacZ Activity
(Relative to WT [PSI+])
ΔNatB
ΔNatC
c
[PSI+]
[psi-]
STRE-mediated LacZ Activity
(Relative to WT [PSI+])
ΔNatB
ΔNatC
d
Hsp104
Sis1
Ssa1/2
Ssb1/2
Protein Accumulation
(Relative to WT [PSI+])
ΔNatB
ΔNatC
ΔNatB
ΔNatC
[PSI+]
[psi-]
e
HSE-mediated LacZ Activity
(Relative to WT [PSI+])
Copy: Low High
Hsf1ON
f
Low Copy
High Copy
Protein Accumulation
(Relative to WT [PSI+])
Hsp104
Ssa1
Sis1
g
Vector
Hsf1DN
ΔNatA
h
WT
ΔNatA
Protein Accumulation
(Relative to WT [PSI+])
Hsf1DN: - + - + - +
Hsp104
Ssa1
Sis1

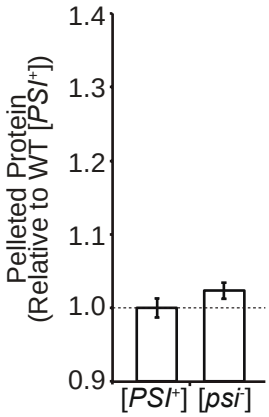
Supplementary Figure 1. a. Lysates from [*PSI*$^{+}$] and [*psi*$^{-}$] WT (SLL2606 and SLL21119, respectively), ΔNatA (SY319 and SY978, respectively), ΔNatB (SY536 and SY563, respectively), and ΔNatC (SY540 and SY567, respectively) were isolated from cultures grown at 30ºC or 37ºC (HS) and analyzed by SDS-PAGE and immunoblotting for Ssa3/4 and Pgk1 (loading control). **b.** Heat shock element (pHSE) activity was analyzed using a β-galactosidase reporter (SB753) expressed in [*PSI*$^{+}$] and [*psi*$^{-}$] ΔNatB (SY536 and SY563, respectively) and ΔNatC (SY540 and SY567, respectively) strains (n=6, *p<0.00001 compared to WT [*PSI*$^{+}$] (error bars represent standard deviation). **c.** Stress response element (pSTRE) activity was analyzed using a β-galactosidase reporter (SB757) expressed in [*PSI*$^{+}$] and [*psi*$^{-}$] ΔNatB (SY536 and SY563, respectively) and ΔNatC (SY540 and SY567, respectively) strains (n=6, *p<0.005 compared to WT [*PSI*$^{+}$] , error bars represent standard deviation). **d.** Expression of Hsp104, Ssa1/2, Sis1, and Ssb1/2 was determined by analyzing lysates of [*PSI*$^{+}$] and [*psi*$^{-}$] ΔNatB (SY536 and SY563, respectively) and ΔNatC (SY540 and SY567, respectively) strains by SDS-PAGE and quantitative immunoblotting using specific antisera (n=6, *p<0.005 compared to WT [*PSI*$^{+}$], error bars represent standard deviation). **e.** pHSE (SB753) reporter activity in strains expressing Hsf1^{ON} from both low copy (cen) and high copy (2μ) vectors, as described in (b) (n=3, p<0.0001, error bars represent standard deviation). **f.** Hsp104, Ssa1/2, and Sis1 protein levels in strains expressing Hsf1^{ON} from low copy (SY2053) and high copy (SY2130) vectors, collected and analyzed as described in (d) (n=4, *p<0.005, error bars represent standard deviation). **g.** pHSE reporter activity in strains ΔNatA (SY319) ± Hsf1^{DN} (SB778) compared to WT [*PSI*$^{+}$], as described in (b) (n=4, p<0.0001, error bars represent standard deviation). **h.** Hsp104, Ssa1/2, and Sis1 protein levels in ΔNatA ± Hsf1^{DN} compared to WT, collected and analyzed as described in (d) (n=5, error bars represent standard deviation).



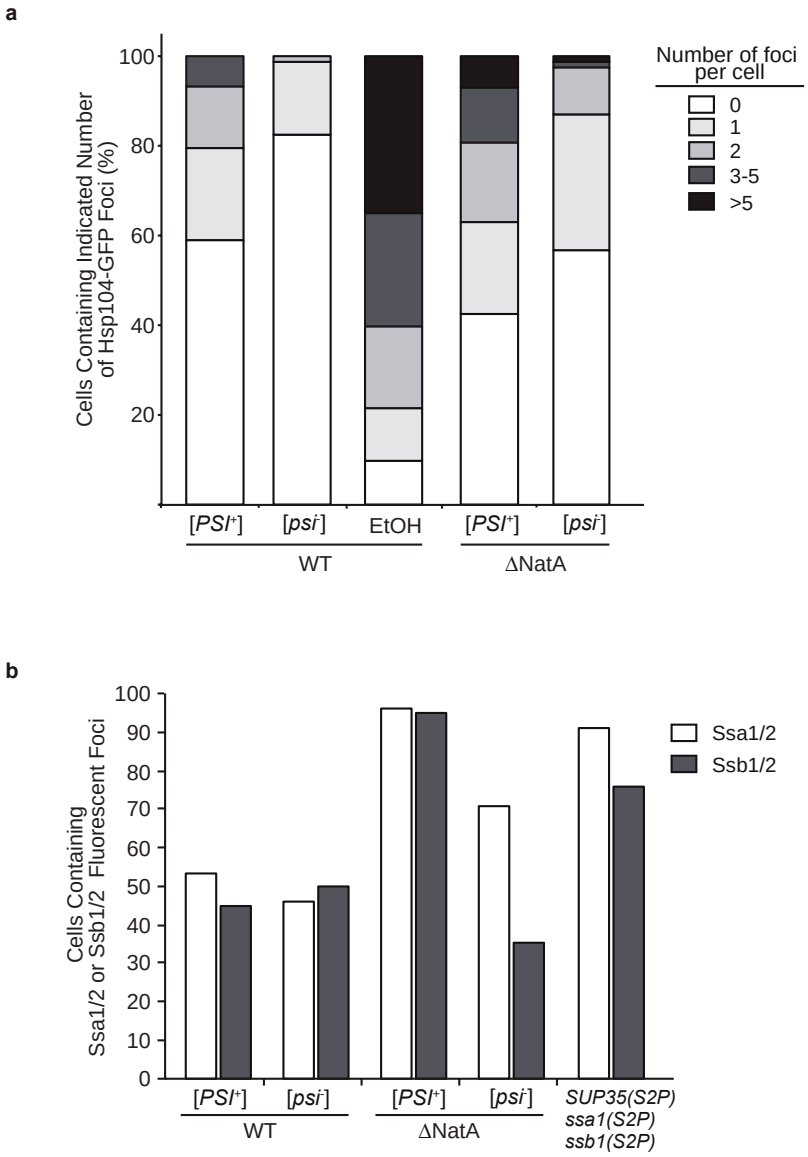
Supplementary Figure 2. Densitometry traces (ImageJ) of SDD-AGE gels shown in **a.** Figure 2d, **b.** Figure 2e, and **c.** Figure 3f.



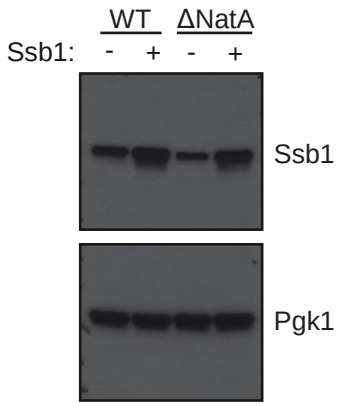
Supplementary Figure 3. a. Growth of [*PSI*$^{+}$] and [*psi*$^{-}$] WT (SLL2606 and SY2119, respectively), ΔNatA (SY319 and SY978, respectively), ΔNatB (SY536 and SY563, respectively), and ΔNatC (SY540 and SY567, respectively), ΔSti1 (strain #s, respectively) strains on YPD supplemented with a gradient up to 100mg/ml hygromycin B, 800 mg/ml paromomycin, and 100 μg/ml cycloheximide **b.** Gene expression clustering for heat shock chaperones, proteins associated with translation, and N-terminal acetylases. Clustering was performed by the Stanford Microarray Database using a previously published dataset.[1]



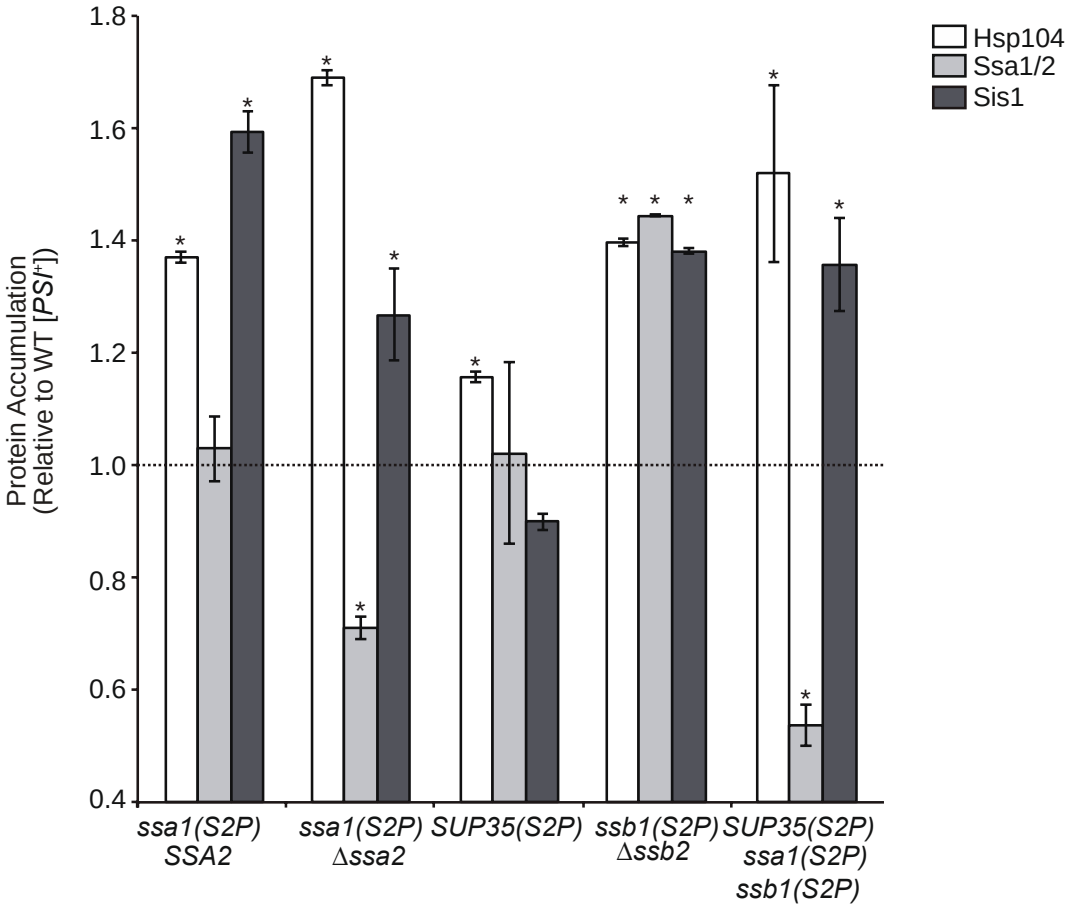
Supplementary Figure 4. Quantification of pelleted proteins in lysates isolated from WT [*PSI*$^{+}$] (SLL2606) and [*psi*$^{-}$] (SLL2119) strains, following centrifugation at 15,000 x *g* (n=3, error bars represent standard deviation).



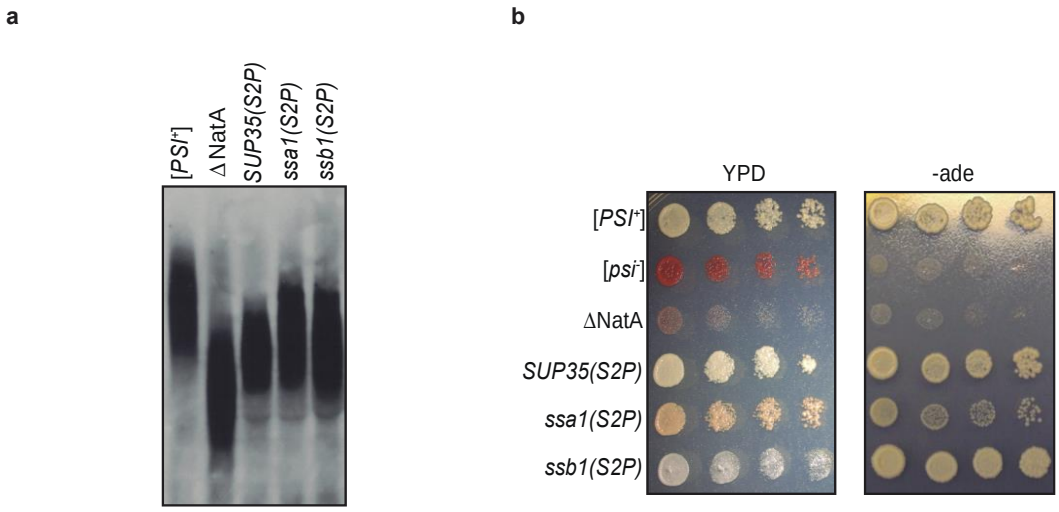
Supplementary Figure 5. a. The number of fluorescent foci per cell was determined in WT [*PSI*$^+$] (SY1906) and [*psi*$^-$] (SY2125), WT [*PSI*$^+$] treated with 7.5% ethanol, and ΔNatA [*PSI*$^+$] (SY2152) and [*psi*$^-$] (SY2183) strains expressing Hsp104-GFP by microscopy (n>150 cells/strain). **b.** The number of cells with visible fluorescent foci was determined in WT [*PSI*$^+$] (SLL2606) and [*psi*$^-$] (SY2119), ΔNatA [*PSI*$^+$] (SY319) and [*psi*$^-$] (SY978) and [*PSI*$^+$] *SUP35(S2P) ssa1(S2P) ssb1(S2P)* (SY2182) strains following fixation and staining for Ssa1/2 (white) and Ssb1/2 (gray) (n ≥ 20 cells).



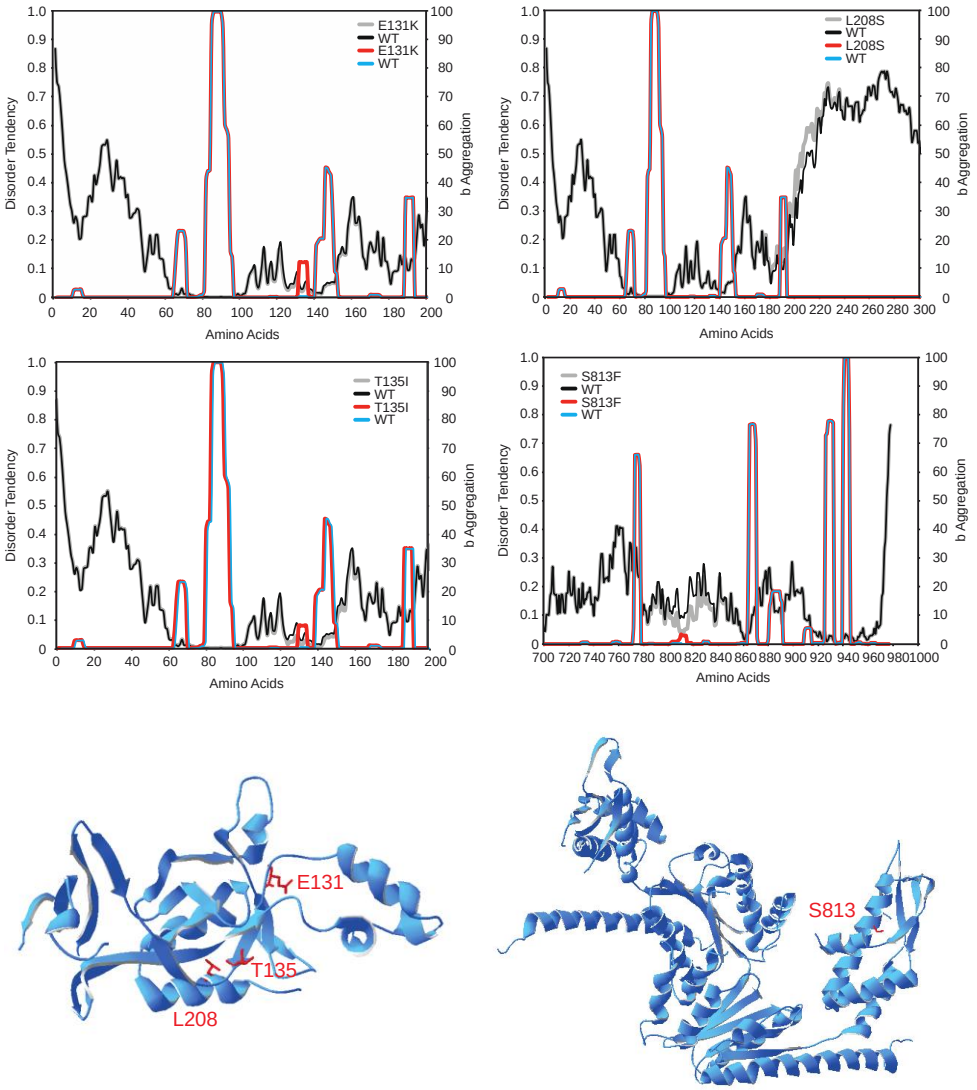
Supplementary Figure 6. SDS-PAGE analysis of lysates isolated from [*PSI*$^{+}$] WT (SLL2606) and ΔNatA (SY319) strains expressing either an empty vector (-) or high copy (2μ) expression plasmid for Ssb1 (SB806). The gels were immunblotted for Ssb1/2 or Pgk1 (loading control) using specific antisera.



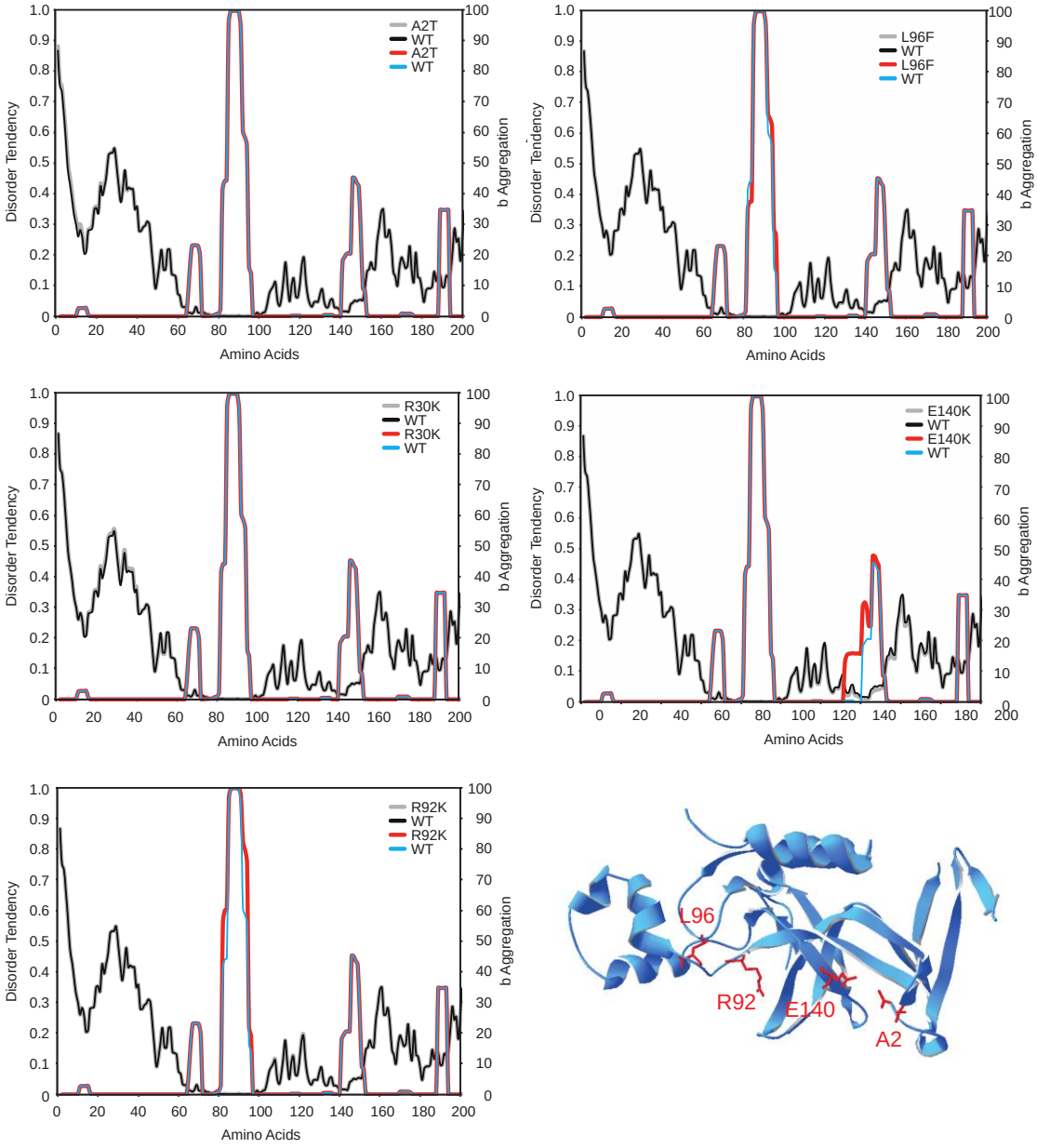
Supplementary Figure 7. Relative levels of Hsp104, Ssa1/2, and Sis1 protein in the [*PSI*$^+$] Δ*ssa*2 (SY1338), *ssa*1*(S2P)* (SY1308), *ssa1(S2P) Δssa2* (SY1339), *SUP35(S2P)* (SY1209), *ssab1(S2P) ΔSSB2* (SY1965), *SUP35(S2P) ssa1(S2P) ssb1(S2P)* (SY2182) strains compared to WT [*PSI*$^+$] (SY2606) as determined by SDS-PAGE and quantitative immunblotting with specific antisera (n=6, *p<0.003, error bars represent standard deviation).



Supplementary Figure 8. a. Lysates isolated from [*PSI*$^+$] strains expressing non-acetylatable (S2P) mutants of *SUP35* (SY1209), *SSA1* (SY1339), or *SSB1* (SY1965) were analyzed by SDD-AGE followed by immunoblotting for Sup35. **b.** 10-fold serial dilutions of cultures of WT ([*PSI*$^+$] and [*psi*$^-$]) (SLL2606 and SLL2119), [*PSI*$^+$] ΔNatA (SY319), [*PSI*$^+$] *SUP35(S2P)* (SY1209), [*PSI*$^+$] *ssa1(S2P)* (SY1339), and [*PSI*$^+$] *ssb1(S2P)* (SY1965), expressing strains were grown on rich medium (YPD) and medium lacking adenine (-ade) at 30ºC.



Supplementary Figure 9. Sir3 mutations that enhance the mating defect of ΔNatA. The Sir3 sequence was analyzed for disorder (IUPred, black) and aggregation potential (Tango, blue) and compared to the disorder (black) and aggregation potential (red) for the indicated Sir3 mutations. The positions of the mutations on Sir3 crystal structures (PDB files 4KUI and 3TE6) are indicated below.[2,3]



Supplementary Figure 10. Sir3 mutations that do not enhance the mating defect of ΔNatA**.** The Sir3 sequence was analyzed as in the legend to Fig. S9. The positions of the mutations on the Sir3 crystal structure (PDB file 4KUI) are indicated below. The R30K is located in a region of the protein that is not present in the structure presumably due to its flexibility.[2]

Supplementary Table 1. Primers

Primer	Sequence
5' Hsf1 KO	5'GAAACAAAAAAGACAAAAAGACAGCTGTATTGTTGGCGCCCAGCTGAAGCTTCGTACG3'
3' Hsf1 KO	5'AAATGATTATATACGCTATTTAATGACCTTGCCCTGTGTAGCATAGGCCACTAGTGGATCTG3'
5' Hsf1 Seq	5'TAACCTAAGGCAAAGGGTTTGTCATATAC3'
3' Hsf1 Seq	5'AAAGTTATGTAATATGCGAATTCTGTTTCTGCTTG3'
PTEF CHK	5'GCACGTCAAGACTGTCAAGG3'
pFA6a	5'TGCCCAGATGCGAAGTTAAGTG3'
5' Hsf1 R206S	5'GAAAATCTTTTATTGTCACGAATAGTGAGGAATTTGTGCACCAAATTTTAC3'
3' Hsf1 R206S	5'GTAAAATTTGGTGCACAAATTCCTCACTATTCGTGACAATAAAAGATTTTC3'
Hsf1 Mid Seq	5'ACAGCCGTCAAGTGGAAC3'
5' pSsb1	5'GCCGAGCTCCATTGCCCTAGATCATCTCATGCATGA3'
3' pSsb1	5'GCCGGATCCTTTGTTCAATTAAAATACTGTAATGATCTTGGGACATC3'
5' Ssb1 WT	5'GCCGGATCCATGGCTGAAGGTGTTTTCCAAGGTGCTATCGGTATCGA3'
5' Ssb1 S2P	5'GCCGGATCCATGCCTGAAGGTGTTTTCCAAGGTGCTATCGGTATCGA3'
3' Ssb1	5'GCCCTCGAGTTAACGAGAAGACATGGCCTTGGTGACAAC T3'
5' Ssb1 Seq	5'TTTTGAACAGATTCGTCGTCGAATCTTCTA3'
3' Ssb1 Seq	5'CCATGCTGCCAAGTATCTTAACAGTCTTT3'
5' Ssb1 KO	5'CAGATGTCCCAAGATCATTACAGTATTTTAATTGAACAAACGGATCCCCGGGTTAATTAA3'
3' Ssb1 KO	5'CAATATAAGTAATATTCATATATATGTGATGAATGCAGTCGAATTCGAGCTCGTTTAAAC3'
5' Ssb2 KO	5'TCGTTTTTTCTTTCAAGAAACCAAGAACCAATATCCTCATTAACACGGATCCCCGGGTTAATTAA3'
3' Ssb2 KO	5'AAAATATATATATGTGTATAACCTTAACCAGAATGACATCGAATTCGAGCTCGTTTAAAC3'
5Sti1pFA6a	5'TCCTCACTGTAGCTACTAAACAACCTATACGCAAGAAAGCAGCTGAAGCTTCGTACGC3'
3Sti1pFA6a	5'AAAAGAATTCAAGATAATAAAGTTATATTTCGTATTATTTGCATAGGCCACTAGTGGATCTG3'

Supplementary Table 2. Shuttle Plasmids

Name	Plasmid	Reference
SB548	pRS306-P$_{SUP35}$-*SUP35(S2P)*	Pezza *et al.* (2009)
SB629	pRS305-P$_{SUP35}$-*SUP35-HA*	DiSalvo *et al.* (2011)
SB732	pRS306-P$_{SSB1}$-*ssb1(S2P)*	This study
SB753	pHSE-lacZ	Liu *et al.* (1998)
SB757	pSTRE-lacZ	Liu *et. al.* (1998)
SB778	pRS315-*EXA3-1*	Halliday and Craig (1995)
SB779	pRS313-P$_{HSF1}$*HSF1*	Halliday and Craig (1995)
SB788	pRS313-P$_{HSF1}$*HSF1(R206S)*	This study
SB806	pRS424-P$_{HSF1}$*HSF1(R206S)*	This study
SB812	pRS426-P$_{HSF1}$*HSF1*	This study
SB814	pRS424-P$_{GPD}$-*SSB1*	This study
SLL6676	pRS316-P$_{SUP35}$-*SUP35*$^{254-685}$	Zhou *et al.* (2001)

Supplementary Table 3. *Saccharomyces cerevisiae* Strains

Strain	Genotype	Reference
74D-694 (SLL2606)	*MATa* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112*	Chernoff *et. al.* (1995)
74D-694 (SLL2119)	*MATa* [*psi-*] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112*	Chernoff *et. al.* (1995)
SY319	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δnat1::hphMX4 Δard1::kanMX4*	Pezza *et. al.* (2009)
SY394	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 ura3-52 his3Δ200 leu2-3, 112 Δssb2::hisMX4 Δssb1::kanMX6*	This study
SY441	*MATα* [*psi*$^-$] *ade1-14 trp1-289 ura3-52 his3Δ200 leu2-3, 112 Δssb2::his3MX4 Δssb1::kanMX6*	This study
SY536	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δnat3::hisMX4*	Pezza *et. al.* (2009)
SY540	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δmak3::hisMX4*	Pezza *et. al.* (2009)
SY563	*MATα* [*psi-*] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δnat3::hisMX4*	Pezza *et. al.* (2009)
SY567	*MATa* [*psi*$^-$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δmak3::hisMX4*	Pezza *et. al.* (2009)
SY978	*MATα* [*psi*$^-$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δnat1::hphMX4 Δard1::kanMX4*	Pezza *et. al.* (2009)
SY1132	*MATa* [*PSI*$^+$]^{weak} *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δnat1::hphMX4 Δard1::kanMX4*	This study
SY1209	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 SUP35(S2P)*	Pezza *et. al.* (2009)
SY1308	*MAT□* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3,112 ssa1(S2P)*	Pezza *et. al.* (2009)
SY1339	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δssa2::hphMX4 ssa1(S2P)*	Pezza *et. al.* (2009)
SY1500	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112::P_{sup35}-SUP35(HA)*	This study
SY1501	*MATα* [*psi*$^-$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112::P_{sup35}-SUP35(HA)*	This study
SY1502	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112::P_{sup35}-SUP35(HA) Δard1::kanMX4*	This study
SY1906	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 HSP104-GFP::kanMX6*	This study
SY1965	*MATα* [*PSI*$^+$] *ade1-14 trp1-289 his3Δ200 leu2-3, 112 Δssb2::hisMX4 Δssb1::kanMX6 ura3-52::P_{ssb1}-ssb1(S2P)*	This study

SY2051	*MAT**a*** [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δhsf1::hphMX4 [CEN P$_{hsf}$-HSF1]*	This study
SY2053	*MAT**a*** [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δhsf1::hphMX4 [CEN P$_{hsf}$-HSF1^{R206S}]*	This study
SY2123	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δssa2::hphMX4 ssa1(S2P) SUP35(S2P)*	This study
SY2124	*MAT**a*** [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 SUP35(S2P) Δhsf1::hphMX4* [*2 micron P$_{hsf}$-HSF1^{R206S}*]	This study
SY2125	*MATα* [*psi*$^{-}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 HSP104-GFP::kanMX6*	This study
SY2129	*MAT**a*** [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 SUP35(S2P) Δhsf1::hphMX4 [2 micron P$_{hsf}$-HSF1]*	This study
SY2130	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δhsf1::hphMX4 [2 micron P$_{hsf}$-HSF1^{R206S}]*	This study
SY2152	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δard1::kanMX6 HSP104-GFP::kanMX6*	This study
SY2153	*MATα* [*psi*$^{-}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112::P$_{sup35}$-SUP35(HA) Δard1::kanMX4*	This study
SY2154	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δard1::kanMX4 SUP35(S2P)*	This study
SY2182	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 leu2-3, 112 Δssb2::hisMX4 Δssb1::KanMX6 Δssa2::hphMX4 ura3-52::P$_{ssb1}$-ssb1(S2P) ssa1(S2P) SUP35(S2P)*	This study
SY2183	*MATα* [*psi*$^{-}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δard1::kanMX6 HSP104-GFP::kanMX6*	This study
SY2195	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 leu2-3, 112 Δssb2::hisMX4 Δssb1::kanMX6 ura3-52::P$_{ssb1}$-ssb1(S2P) SUP35(S2P)*	This study
SY2348	*MATα* [*PSI*$^{+}$] *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112 Δsti1::kanMX6*	This study
SLL2600	*MAT**a*** [*PSI*$^{+}$]^{weak} *ade1-14 trp1-289 his3Δ200 ura3-52 leu2-3, 112*	Derkatch *et al.* (1999)

Supplementary Table 4. Two-sample Kolmogorov-Smirnov tests on lengths of disordered regions

	By NatA	By NatB	By NatC
Not acetylated	D = 0.08; $p = 2\times10^{-6}$	D = 0.15; $p = 2\times10^{-15}$	D = 0.19; $p = 6\times10^{-9}$
By NatA		D = 0.10; $p = 8\times10^{-6}$	D = 0.12; $P = 2\times10^{-3}$
By NatB			D = 0.14; $p = 4\times10^{-4}$

Supplementary References

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2 Yang, D. *et al.* Nalpha-acetylated Sir3 stabilizes the conformation of a nucleosome-binding loop in the BAH domain. *Nature Struct Mol Biol* **20**, 1116-1118 (2013).

3 Ehrentraut, S. *et al.* Structural basis for the role of the Sir3 AAA+ domain in silencing: interaction with Sir4 and unmethylated histone H3K79. *Genes Dev* **25**, 1835-1846 (2011).