Supplemental Figure Legends

Figure S1. Loss of NatA function does not alter the [*URE3*] phenotype. Wildtype (WT, NT64) and Δ*ard1* [*URE3*] (+, SY1444) or [*ure-o*] (-) strains were spotted on rich media (YPD) or media lacking adenine (SD-ade) to monitor the [*URE3*] phenotype (adenine prototrophy).

Figure S2. *sup35K556E* accumulates to a wildtype level. Yeast lysates from a wildtype (WT, 74D-695) or *sup35K556E* (*K556E*, TRS64) were analyzed by SDS-PAGE and immunoblotting for Sup35 or Pgk1, as a loading control. The altered migration of Sup35K556E likely results from the change in charge due to the mutation.

Figure S3. Stop codon readthrough in wildtype and Δ*nat1* heterozygous mutants. Shown are brightfield (DIC) and fluorescent (DsRed) images of crosses between a [*psi*$^-$] strain expressing GST-UGA-DsRED-NLS (SY1181) and wildtype [*PSI*$^+$], wildtype [*psi*$^-$], or Δ*nat1* [*PSI*$^+$] (SY356) strains. The decreased level of readthrough in the heterozygous mutant indicates that loss of one copy of *NAT1* is sufficient to alter the [*PSI*$^+$] phenotype, although readthrough is still detected relative to the [*psi*$^-$] control.

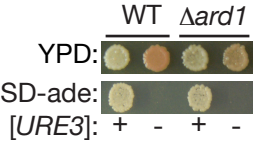
Figure S4. Disruption of NatA does not alter chaperone expression profiles. Yeast lysates from a wildtype yeast strain (74D-694) grown at 30°C (WT) or at 37°C (HS) or from mutant strains Δ*ard1* (TRS169, TRS220), Δ*nat1* (SY356, SY357),

Δ*ard1*Δ*nat1* (SY319, SY978) or Δ*hsp104* grown at 30°C were analyzed by SDS-PAGE and immunoblotting for Hsp104, Ssa1/2, Ssa3/4 or Pgk1, as a loading control.

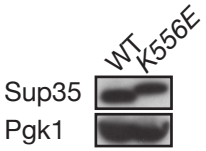
Figure S5. Ssa3/4 expression is not induced in *ssa1S2P* or *ssa2S2P*Δ*ssa2* strains. Yeast lysates from a wildtype strain grown at 30°C (WT) or at 37°C (HS) and from a *ssa1S2P* (*S2P*, SY1308) and a *ssa1S2P*Δ*ssa2* (*S2P* Δ*2*, SY1339) grown at 30°C were analyzed by SDS-PAGE and immunoblotting for Ssa3/4.

Figure S6. Loss of NatA function does not alter the number of [*PSI*$^+$] propagons. Wildtype (white circles) and Δ*ard1*Δ*nat1* (black circles, SY319) [*PSI*$^+$] strains were treated with GdnHCl over time in exponentially growing cultures and analyzed for the percentage of [*PSI*$^+$] cells remaining at the indicated number of generations as described in the methods.

Figure S7. Expression of GST from P_{GPD}GSTUGADsREDNLS is identical in wildtype and Δ*nat1* strains. Lysates from a wildtype [*psi*$^-$] (*NAT1*) strain or from wildtype [*psi*$^-$] (*NAT1*, SY1181) or Δ*nat1* (SY1180) strains expressing GSTUGADsREDNLS (+) were analyzed by SDS-PAGE and immunoblotting for GST or Pgk1, as a loading control. The similar accumulation of GST in wildtype and mutant strains indicates that expression of the reporter was not directly affected by loss of NatA function.



Pezza, Langseth et al.
Figure S1



Pezza, Langseth et al.
Figure S2

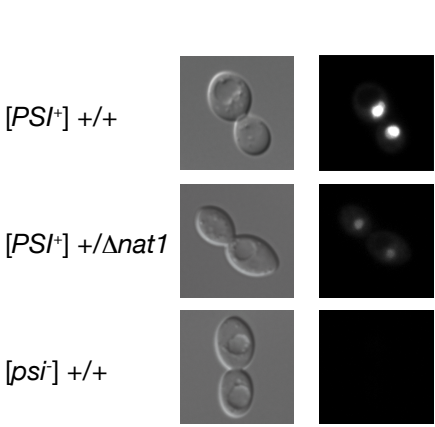


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Figure S3

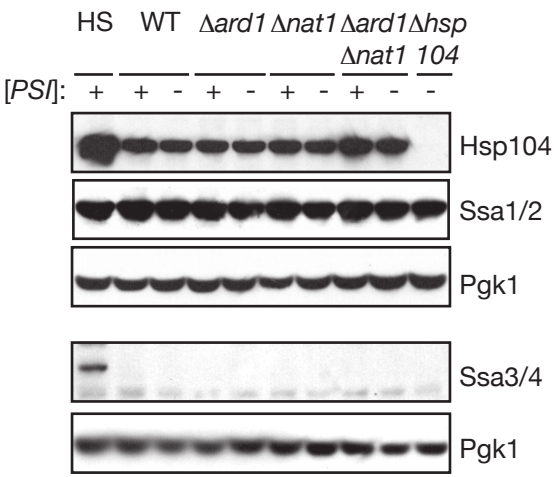

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Figure S4

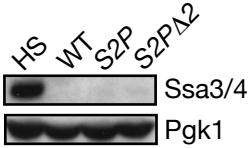

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Figure S5

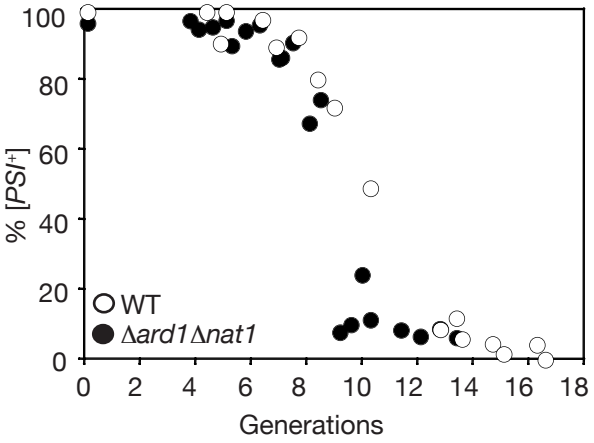

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Figure S6

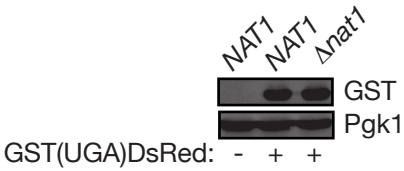

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Figure S7