Albanèse et al., http://www.jcb.org/cgi/content/full/jcb.201001054/DC1

Figure S1. **The ZHD domain is conserved between Zuo1 and Jjj1.** Alignment of the ZHD domain region of Jjj1 and Zuo1 homologues from different yeast species reveals highly conserved residues. The alignment, performed with the Clustal software, highlights the regions of conservation (circles) and homology (asterisks).

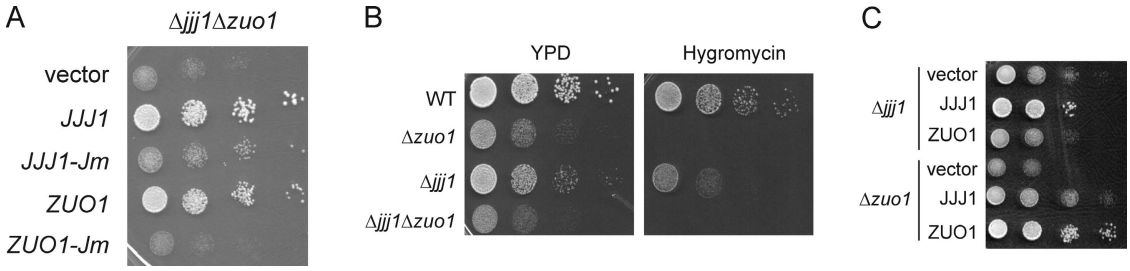

Figure S2. **Phenotypes of Jjj1 and Zuo1 expression in *Δjjj* and *Δzuo1* cells.** (A) Requirement of functional J domains in Jjj1 and Zuo1 for complementation of *Δjjj1Δzuo1* phenotype. Cells were grown overnight in −URA at 30°C, and an equal number of cells was spotted as a 10-fold dilution series on −URA plates and incubated at 30°C for 2 d. (B) Cells deleted for *ZUO1* but not *JJJ1* are hypersensitive to hygromycin. Cells were grown overnight in YPD at 30°C, and an equal number of cells was spotted as a 10-fold dilution series on YPD or YPD + 50 µg/ml hygromycin plates and incubated at 30°C for 2 d. (C) Overexpression of Jjj1 can suppress the slow growth phenotype of Δ*zuo1*, but overexpression of Zuo1 cannot suppress the Δ*jjj1* phenotype. An equal number of cells was spotted as a 10-fold serial dilution on −URA plates and incubated at 30°C for 2 d. Vector, cells transformed with an empty p426 vector.

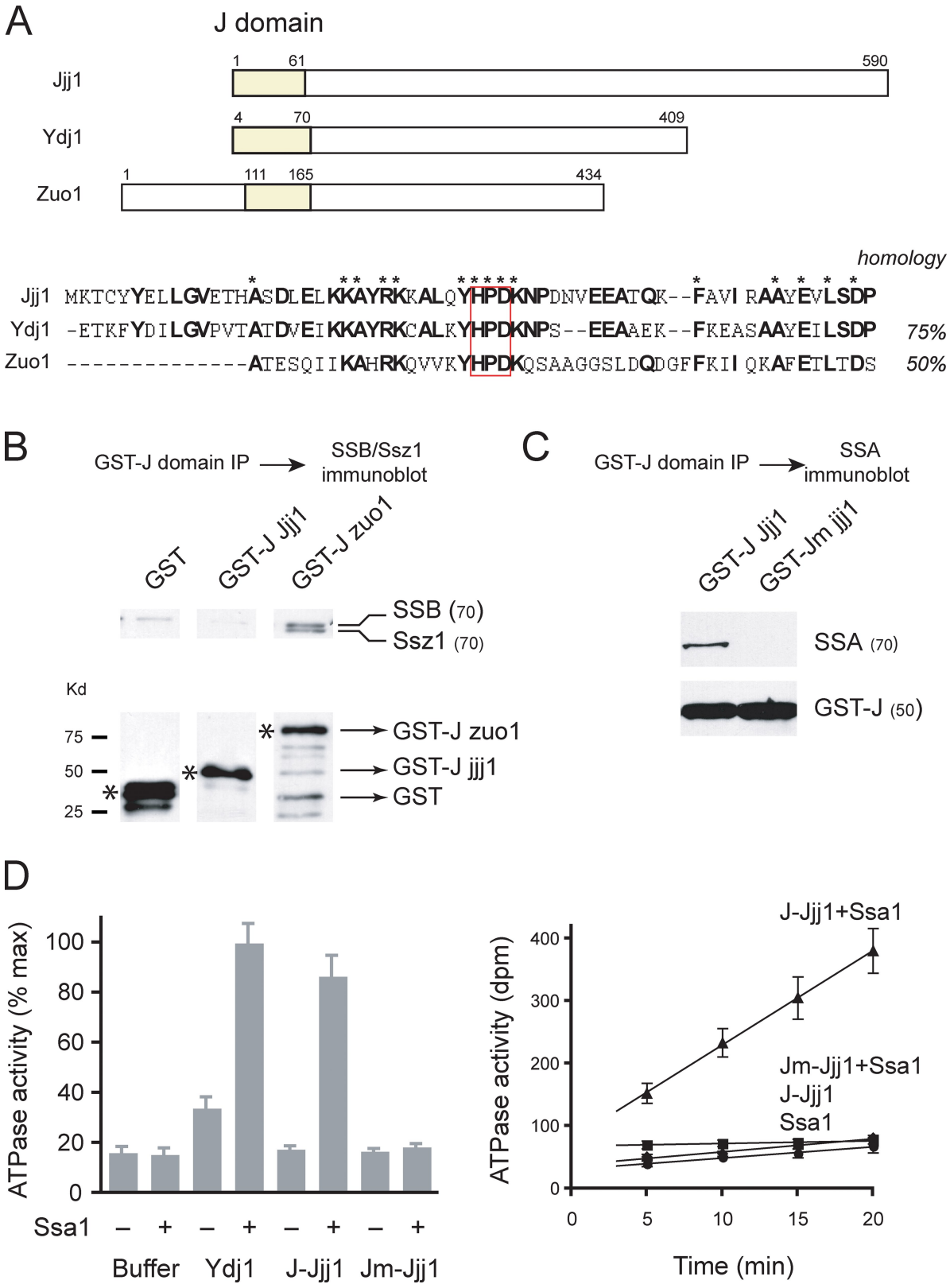

Figure S3. **J domains of Zuo1 and Jjj1 suffice to confer specificity for different Hsp70s.** (A) Clustal alignement of J domains of Jjj1, Ydj1, and Zuo1. The red box represents the canonical HPD motif. Asterisks indicate an amino acid conservation in the alignment. (B) The J domains of Zuo1 and Jjj1 by themselves can interact with different classes of cytosolic Hsp70s. The J domain of Jjj1 and the N terminus plus J domain of Zuo1 were fused to GST and expressed in yeast. After GST isolation, bound proteins were separated on SDS-PAGE an analyzed by immunoblotting using anti-SSB, anti-Ssz1, and anti-GST antibodies. Asterisks indicate migration of GST-fusion protein. (C) Purified GST carrying the Jjj1 J domain or the Jjj1 Jm domain carrying the HPD mutation was incubated with yeast-purified SSA. After GST purification, the proteins were analyzed by immunoblotting using anti-SSA antibody. IP, immunoprecipitation. (D) The J domain of Jjj1 by itself can stimulate the ATPase activity of SSA. SSA–ATP[^{32}P] complexes were incubated with the J domain of Jjj1, with Jm, which is the J domain with a point mutation in the HPD motif, or with purified Ydj1. The rates of hydrolysis (left) were deduced from the kinetics of the reaction (right). The mean and standard error of at least three independent experiments is shown.

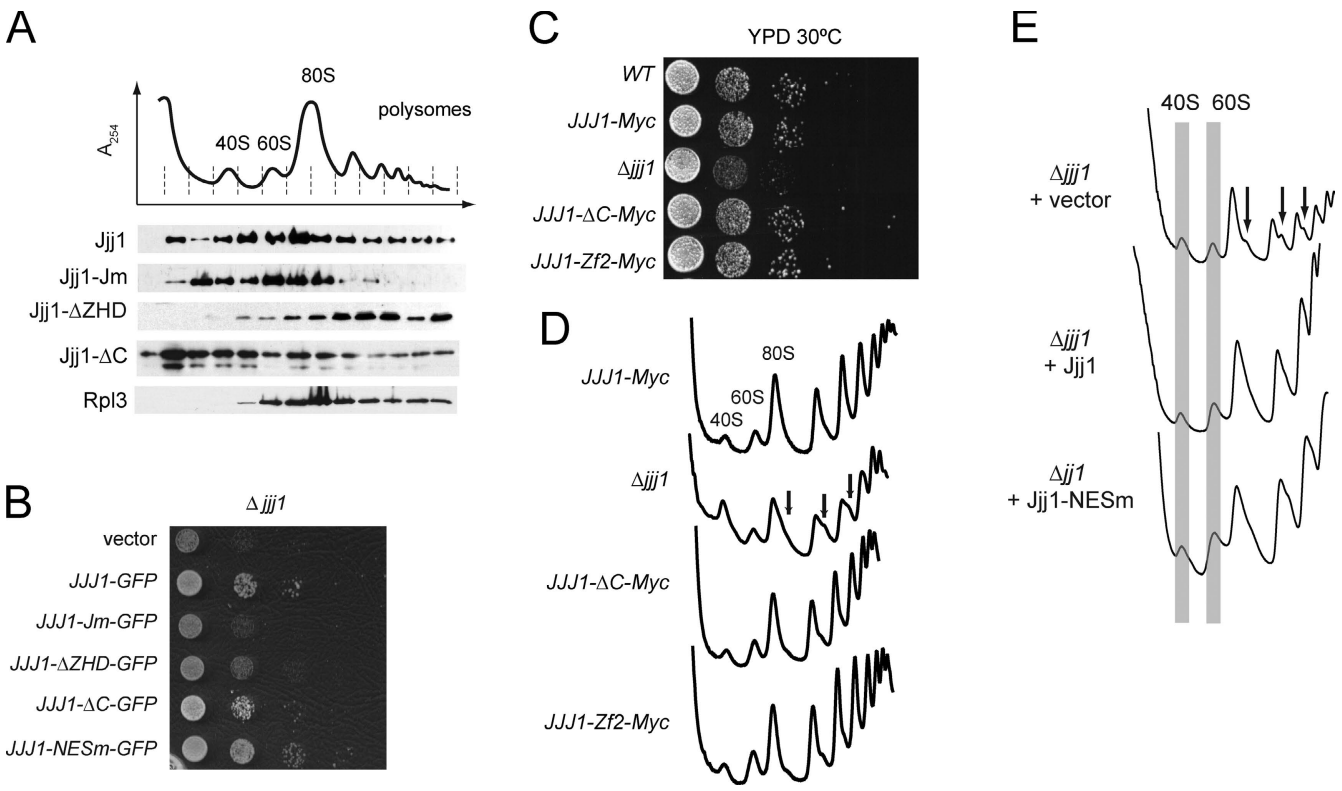

Figure S4. **Ribosome association and biological function of Jjj1 and Jjj1 domain mutants is independent of expression levels.** (A) Sucrose gradient analysis of the association of Jjj1 or Jjj1 variants with ribosomal particles after expression from a high copy number plasmid. The same result for the centromeric plasmid is shown in Fig. 4 B, and results for the chromosomally integrated Jjj1-ΔC and Jjj1-ΔZf2 are shown in Fig. 4 B. (B) In vivo functionality of Jjj1 and Jjj1 mutants fused to GFP and assessed by their ability to complement *Δjjj1* cells. Cells were grown overnight at 30°C, and an equal number of cells was spotted as a 10-fold dilution series on −URA plates and incubated at 30°C for 2 d. Note the similarity of in vivo rescue phenotype for Jjj1-GFP variants with those observed for Jjj1 lacking the GFP moiety (Fig. 5 A). (C) Rescue of the *Δjjj1* slow growth phenotype by Jjj1 variants integrated at its chromosomal locus. (D) Deletion of the C-terminal domain or the Zf2 domain from the chromosomal copy of Jjj1 does not affect polysome profiles. Yeast lysates were fractionated on a 7–47% sucrose gradient, and the $OD_{254\ nm}$ was monitored. The positions of 80S ribosomes and 40S and 60S ribosomal subunits are indicated. The arrows indicate the presence of halfmers. (E) Rescue of *Δjjj1* aberrant polysome profile by Jjj1-NESm. Yeast lysates were fractionated on a 7–47% sucrose gradient, and the OD_{254} was monitored. The gray columns indicate the 40S and the 60S peaks. The arrows indicate the presence of halfmers containing extra 48S initiation complexes.

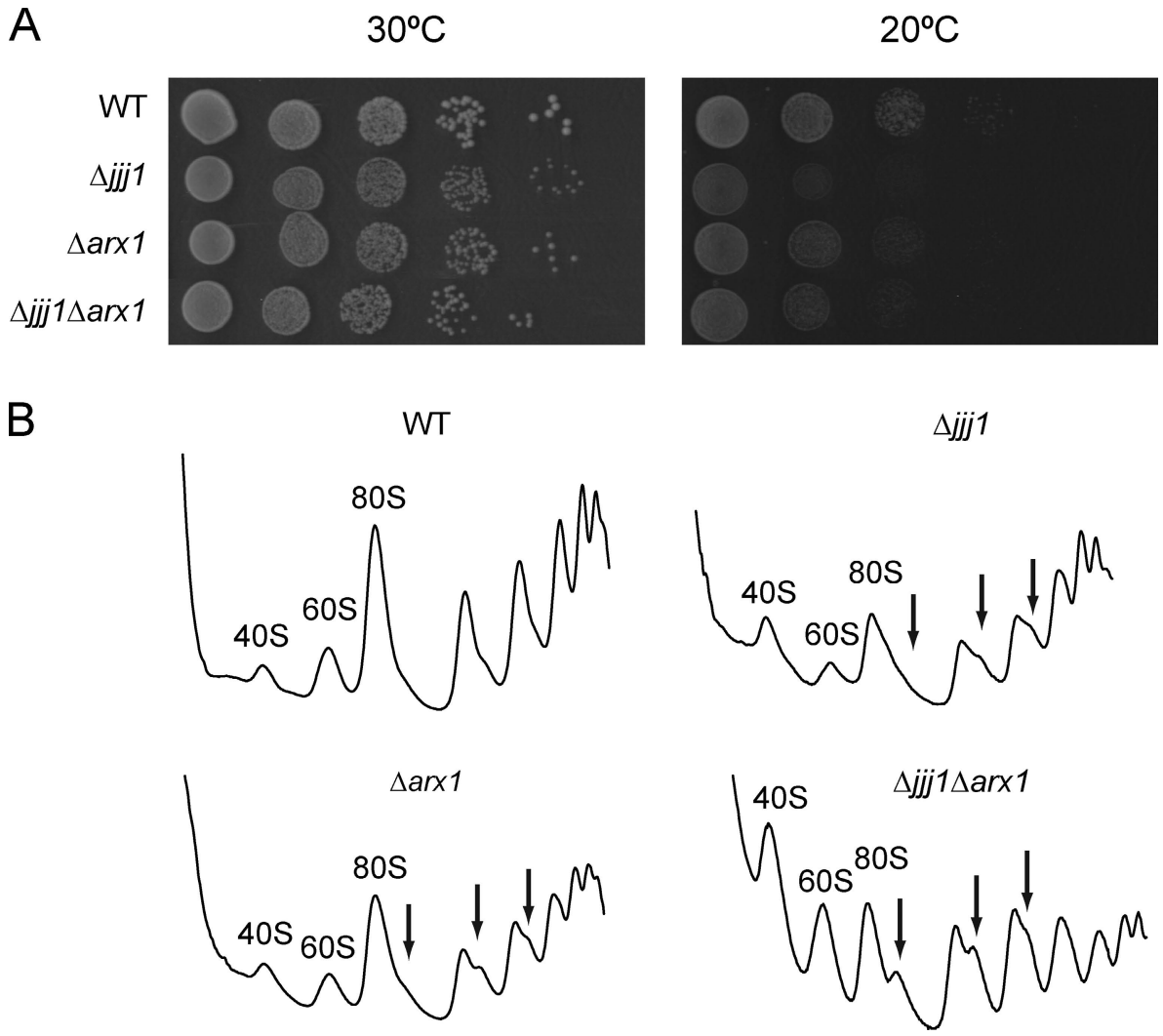

Figure S5. **Deletion of Arx1 rescues the cold sensitivity of *Δjjj1* cells but not the ribosome biogenesis defect.** (A) Cells were grown overnight in YPD at 30°C, and an equal number of cells was spotted as a 10-fold dilution series on YPD. (B) 20 $OD_{254\,nm}$ yeast lysates from WT, *Δjjj1*, *Δarx1*, and *Δjjj1Δarx1* were fractionated on a 7–47% sucrose gradient and the $OD_{254\,nm}$ monitored. The positions of 80S ribosomes and 40S and 60S ribosomal subunits are indicated. Arrows indicate the half-mers. Association of endogenous Arx1-TAP with ribosomal particles or with polysomes was assessed in the indicated deletion strains by sucrose gradient sedimentation followed by immunoblotting. Association of Arx1 with the denser polysome fractions is indicative of a failure to recycle Arx1 in the nucleus.

Table S1. **CLIPS chaperones analyzed in this study and previously proposed functions**

Ribosome-associated CLIPS chaperone	Domains	Proposed function	Reference
JJJ1	J domain ZDH K/R-charged domain Two zinc fingers	Binds to REI1 Assists recycling of shuttling factor ARX1 to nucleus	Demoinet et al., 2007; Meyer et al., 2007
ZUO1	J domain ZHD K/R-charged domain	Binds to ribosomes through its charged domain Binds Hsp70 SSZ1 to form RAC, stimulating ATPase of SSB	Yan et al., 1998 Huang et al., 2005
SSZ1	Hsp70 ATPase domain	Binds to ZUO1 to form RAC, stimulating SSB ATPase activity	Huang et al., 2005 Gautschi et al., 2001
SSB1/2	Hsp70 ATPase domain	Hsp70, ATPase that binds to nascent chains	Huang et al., 2005 Albanèse et al., 2006
SSA (homologues1–4)	Hsp70 ATPase domain	Major Hsp70 in cytosol and nucleus Binds polysomes and nascent chains Other functions (organelle import, stress response, etc.)	Frydman, 2001 Hartl et al., 2009

Table S2. **Selected features and functions of ribosome biogenesis factors used in this study**

Ribosome biogenesis factors	Domain	Localization	Function
NOC1	CBF	Nucleolus, nucleus	Constituent of 66S pre-ribosomal particles
NUG1	GTPase domain	Nucleolus, nucleus	Associates with nuclear 60S preribosomes, required for export of 60S subunits from the nucleus
NSA3	Ribosomal protein L7AE like	Nucleolus, cytoplasm	Not determined but binds to 66S preribosomal particles
NOG2	GTPase domain	Nucleolus, nucleus	Associates with pre-60S ribosomal subunits in the nucleolus; involved in nuclear export and maturation
Exosome	oligomeric nuclease complex	Cytoplasm, nucleolus, nucleus	Exonuclease involved in rRNA processing
ECM1	ND	Nucleolus, nucleus	Not determined; 60S ribosome biogenesis
ARX1	ND	Predominantly nuclear, shuttles to the cytoplasm	Shuttling factor for pre-60S; participates in 60S ribosomal export
NMD3	ND	Cytoplasm, shuttles from the nucleus to the cytoplasm	Shuttling factor; nuclear export of the 60S ribosomal subunit
REI1	three C2H2 zinc fingers	Cytoplasm	Cytoplasmic recycling of shuttling factors Alb1, Arx1, and Tif6 at the end of 60S biogenesis
LSG1	GTPase domain	Cytoplasm	Helps release Nmd3 from cytoplasmic 60S

For reviews see Fromont-Racine et al. (2003), Tschochner and Hurt (2003), and Strunk and Karbstein (2009).

Table S3. **Summary of function of different domains in JJJ1**

Function	JJJ1	JJJ1-ΔZHD	JJJ-Jm	JJJ1-ΔC
Subcellular localization	Cytoplasmic	Cytoplasmic	Cytoplasmic	Nucleolar/nuclear
Ribosome/polysome association	60S, 80S, and polysomes	Mostly polysomes	Mostly 60S and 80S	60S; no binding to polysomes
Suppression of half-mers in *Δjjj1* cells	Yes	No	No	Yes
Hsp70 activation	Yes	ND	No	ND
Rei1 binding	Yes	Yes	Yes	No
Recycling of Arx1 into the nucleus	Yes	No	No	Yes
Suppression of the slow growth of *Δjjj1* cells	Yes	No	No	Yes
Suppression of slow growth of *Δssb1/2* cells	Yes	No	No	Yes
Suppression of slow growth of *Δzuo1* cells	Yes	No	No	Yes

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