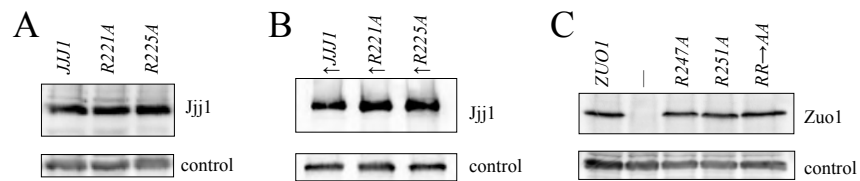


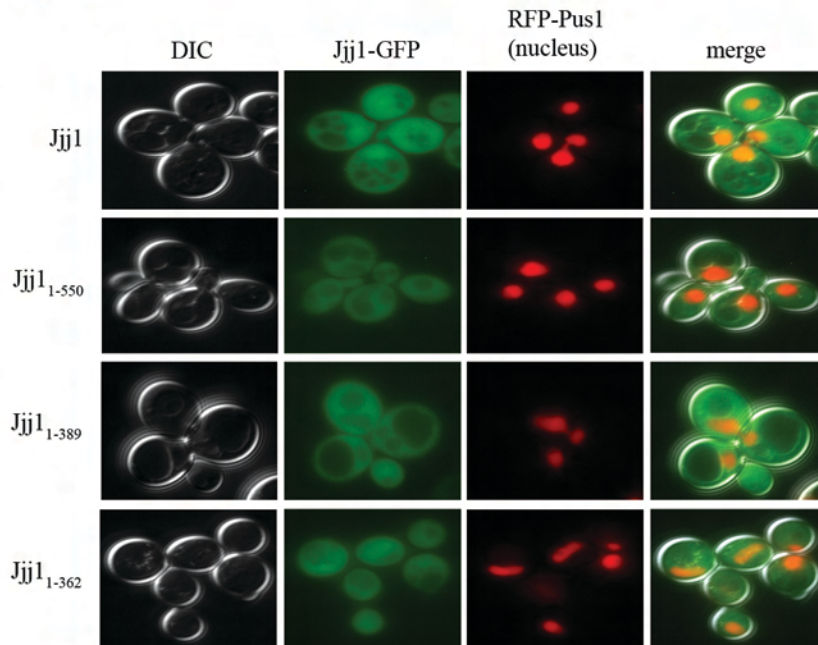
Zuo1	M F S L P T L T S D I T V E V N S S A T K T P F V R R R V E P V G K F F L Q H A Q R T L R N H T W S E F E R I	55
DNAJC2	M L L L P S A A D G R G T A I T H A L T S A S T L C Q - V E P V G R W F E A F V K R R N R - N A S A S F Q E L	53
Jj1	- -	50
DNAJC21	- -	0
Zuo1	E A E K N V K T V D E S N V D P D E L L F D T E L A D E D L - - L T H D A R D W K T A D L Y A A M G L S K L	107
DNAJC2	E D K K E L S E E S E - - - - - - - - - - D E E L Q L E E F P M L K T L D P K D W K N Q D H Y A V L G L G H V	98
Jj1	- -	11
DNAJC21	- -	10
Zuo1	R F R A T E S Q I I K A H R K Q V V K Y H P D K Q S A A G G S L D Q - - D G F F K I I Q K A F E T L T D S N K	160
DNAJC2	R Y K A T Q R Q I K A A H K A M V L K Y H P D K R K A A G E P I K E G D N D Y F F T C I T K A Y E M L S D P V K	153
Jj1	E T H A S D L E L K K A Y R K K A L Q Y H P D K N P D N V E E A T Q K - - - F A V I R A A Y E V L S D P Q E	62
DNAJC21	R R D A S E E E L K K A Y R K L A L K W H P D K N L D N A A E A A E Q - - - F K L I Q A A Y D V L S D P Q E	61
Zuo1	R A Q Y D -	177
DNAJC2	R R A F N -	170
Jj1	R A W Y D S H K E Q I L N D T P P S T D D Y Y D Y E V D A T V T G V T T D E L L L F F N S A L Y T K I D N S A	117
DNAJC21	R A W Y D N H R E A L L -	104
Zuo1	K G - T D Y D F Y E A W G P V F E A E A R F S K K -	201
DNAJC2	K S E A K D N F F E V F T P V F F E A E R N S R W S N K -	195
Jj1	A G - - - - - I Y Q I A G K I F A K L A K D E I L S G K R L G K F S E Y Q D D V F E Q D I N S I G Y L K A C D	167
DNAJC21	K G - - - - - F Y T V Y R N V F E M I A K E E L E S -	125
Zuo1	- - - - - - - T P I P S L G N K D S S K K - E V E Q F Y A F W H R F D S W R T F E F L D E D V P D D S S N R	247
DNAJC2	- - - - - - - K N V P K L G G D M N S S F E - D V D I F Y S F W Y N F D S W R E F S Y L D E E E K E K A E C R	241
Jj1	N F I N K T D K L L Y P L F G Y S P T D Y E - Y L K H F Y K T W S A F N T L K S F S W K D R E Y M Y S K N Y D R	221
DNAJC21	- - V L E E E V D F P T F G D S Q S D Y D T V V H P F Y A Y W Q S F C T Q K N F A W K E E Y D T R Q A S N R	178
Zuo1	D H K R Y I E R K N K A A R D K K K T A D N A R L V K L V E R A V S E D P R I K M F K E E E K K E K E R R K W	302
DNAJC2	D E R R W I E K Q N R A T R A Q R K K E E M N R I R T L V D N A Y S C D P R I K K F K E E E K A K K - - - - -	291
Jj1	R T K R E V N R R N E K A R Q Q A R N E Y N K T V K R F V V F I K K L D K R M K E G A K I A E E Q R - - - - -	271
DNAJC21	W E K R A M E K E L N K K I R D K A R K E K N E L V R Q L V A F I R K R D K R V Q A H R K L V E E Q N - - - - -	228
Zuo1	E R E A G A R A E A E A K A K A E A E A K A K A E S E A K A N A S A K A D K -	341
DNAJC2	E A E K K A K A E A K R K E Q E A K E K Q R K N A E L E A A R L A K E K E E E -	339
Jj1	- -	307
DNAJC21	- - - - - - - A E K A R K A E E M R R Q Q K L K Q A K L V E Q Y R E Q S -	266
Zuo1	K A K E A A K A A K K N K R A I R N S A K E A D Y F G - D A D K A T T I D E Q V G L I V D S L N - - - - -	389
DNAJC2	K E K D I Q K K A I K K E R Q K L R N S C K T W N H F S D N E A E R V K M M E E V E K L C D R L E L A S L Q C	394
Jj1	- - - - - - - E E - - - N W D E L E K V Y D N F G - E F E N S K N D K E G E V L I Y E C F I C N - - - - -	345
DNAJC21	E L Q E M E A R Y E K E F G D G S D E N E M E E H E L K D E E D G K D S D E A E D A E L Y D D L Y C P A C D K	321
Zuo1	- -	413
DNAJC2	L N E T L T S C T K E V G K A A L E K Q I E E I N E Q I R K E E N -	435
Jj1	T F K S E K Q L K N H I N T K L H K K N M E E I R K E M E E E N I T L G L D N L S D L E K F D S A D E S V K E	400
DNAJC21	S F K T E K A M K N H E K S K K H R E M V A L L K Q Q L E E E E E N F S R P Q I D E N P L D D N S E E E M E D	376
Zuo1	S A K T I V D S G K -	426
DNAJC2	A S K N T E K S T G G G G N G S K N W S E D D L Q L L I K A V N L F P A G -	472
Jj1	K E D I D L Q A L Q A E L A E I E R K L A E S S S E D E S E D D N L N I E M D I E V E D V S S D E N V H V N T	455
DNAJC21	A P K Q K L S K K Q -	425
Zuo1	- -	433
DNAJC2	- -	517
Jj1	K N K K K R K K K K K A K V D T E T E E S E S F D D T K D K R S N E L D D L L A S L G D K G L Q T D D D E D W	510
DNAJC21	E S E H K C A K M L L E N R Q N Y D D N F N V N G P G E G V K V D P E D T N L N Q D S A K E L E D S P Q E N V	480
Zuo1	- -	433
DNAJC2	N K - - - K A F D K F K K E H G V V P Q A D N A T P S E R F E G P Y T D F T P W T T E E Q K L L E Q A L K T	568
Jj1	S T - - - K - - - - - A K K K K G K Q P K K N S K S T K S - - - - - T P S L S - - - - - - - - - - - - - - - - -	536
DNAJC21	S V T E I I K P C D D P K S E A K S V P K P K G K T K -	512
Zuo1	- -	433
DNAJC2	Y P V N T P E R W E K I A E A V P G R T K K D C M K R Y K E L V E M V K A K K A A Q E Q V L N A S R A K K	621
Jj1	- - - T L P S S M S P T S A I E V C T T C G E S F D S R N K L F N H V K I A G H A A V K N V V K R K K V K T K	588
DNAJC21	- P V R V P A E P Q T M S V L I S C T T C H S E F P S R N K L F D H L K A T G H A R A P S S S S L N S A T S S	566
Zuo1	- -	433
DNAJC2	- -	621
Jj1	R I	590
DNAJC21	Q S K K E K R K N R	576

Supplementary Fig. 1. Zuo1 and Jj1 share a common region in addition to the J-domain. Sequence alignment of *S. cerevisiae* Zuo1, Jj1 and their *H. sapiens* homologs (DNAJC2 and DNAJC21, respectively) was done with ClustalW; identical residues are boxed. The conserved residues in the J-domain and Zuotin homology domain (ZHD) are indicated; the boundaries are arbitrarily set at the first and last identical residue in each region.

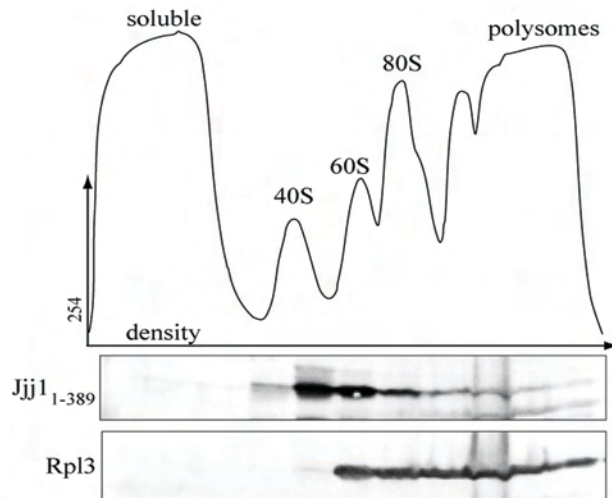


Supplementary Fig. 2. Expression levels of Zuo1 and Jjj1 variants in cell lysates. (A-C) Cell extracts were prepared from cultures of $\Delta zuo1$ or $\Delta jjj1$ cells expressing the indicated protein, and subjected to immunoblot analysis using antibody specific to either the Jjj1 C-terminus or to Zuo1, as well as antibody specific to Ssc1 as a loading control. A) $\Delta jjj1$ cells containing plasmid encoding WT *JJJ1* or the indicated *JJJ1* mutant; extracts prepared from cultures used for serial dilutions shown in Fig. 1C. B) $\Delta zuo1$ cells containing plasmid encoding WT *JJJ1* or the indicated *JJJ1* mutant expressed from the *GPDI* promoter (\uparrow); extracts prepared from cultures used for serial dilutions shown in Fig. 1D. C) $\Delta zuo1$ cells containing plasmid encoding WT *ZUO1*, no insert (—) or the indicated *ZUO1* mutant (Zuo1_{R247,251A} indicated by RR \rightarrow AA); extracts prepared from cultures used for serial dilutions shown in Fig. 1E.

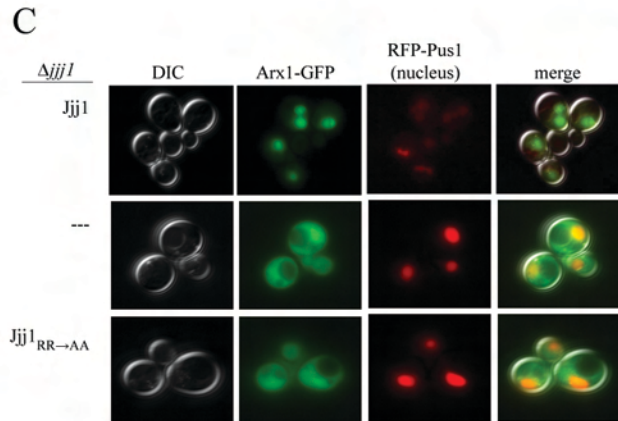
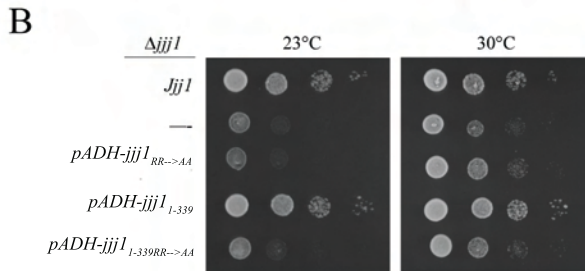
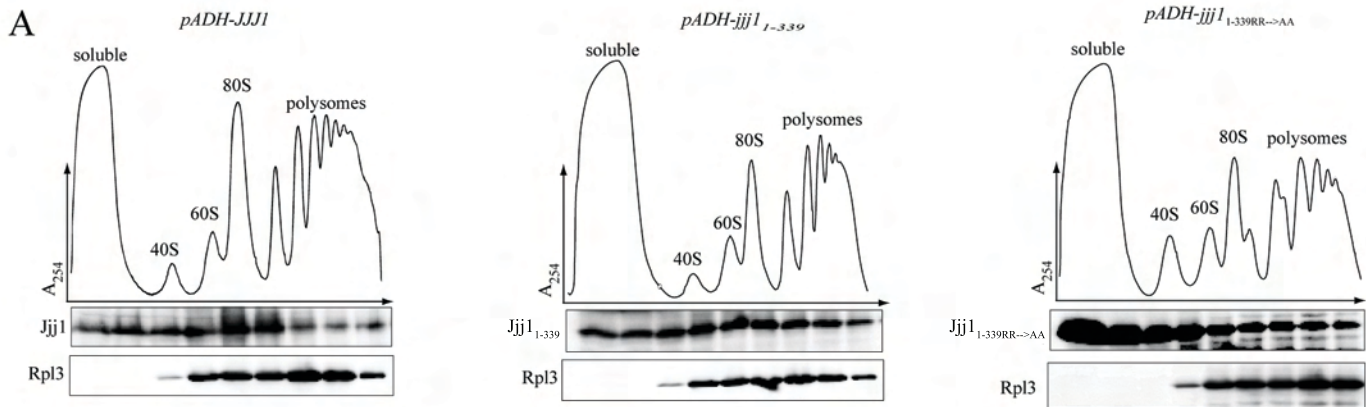
A



B



Supplementary Fig. 3. Analysis of cellular localization of Jjj1 C-terminal truncations. A) $\Delta j j j 1$ cells expressing either Jjj1-GFP, Jjj1₁₋₅₅₀-GFP, Jjj1₁₋₃₈₉-GFP or Jjj1₁₋₃₆₂-GFP from the native promoter, and the nucleus-specific protein RFP-Pus1 as a nuclear marker, were grown at 30°C prior to imaging by fluorescence and differential interference contrast (DIC) microscopy. Representative images show DIC, localization of Jjj1-GFP, Jjj1₁₋₅₅₀-GFP, Jjj1₁₋₃₈₉-GFP or Jjj1₁₋₃₆₂-GFP as indicated, localization of RFP-Pus1 and an image overlay (merge). B) Lysates from $\Delta j j j 1$ cells expressing Jjj1₁₋₃₈₉ from the native promoter was centrifuged through a sucrose gradient to separate ribosomal subunits, monosomes and polysomes and fractions were collected. Upper, absorbance at 254 nm plotted versus the relative time of fraction collection (density). Lower, fractions were analyzed by immunoblotting using antibodies specific for the N-terminus of Jjj1 and Rpl3.



Supplementary Fig.4. A,B) Overexpression of Jjj1 variants. A) Lysate from $\Delta jjj1$ cells expressing either wild-type Jjj1, Jjj1₁₋₃₃₉ or Jjj1_{1-339RR->AA} from the *ADH1* promoter (*pADH*) was centrifuged through a sucrose gradient to separate ribosomal subunits, monosomes and polysomes and fractions were collected. Upper, absorbance at 254 nm plotted versus the relative time of fraction collection (density). Lower, fractions were analyzed by immunoblotting using antibodies specific for the N-terminus of Jjj1 and Rpl3. B) $\Delta jjj1$ cells containing plasmid encoding WT *JJJ1*, or the indicated *JJJ1* mutant expressed from the *ADH1* promoter (*pADH*), were serially diluted, spotted on minimal medium plates, and then incubated at 23°C for 3 days or 30°C for 2 days. C) Analysis of Arx1 localization in *jjj1_{RR->AA}* cells. $\Delta jjj1$ *ARX1-GFP* cells containing empty vector (---), or expressing Jjj1 or Jjj1_{RR->AA} from the native promoter, were grown to an OD₆₀₀ of 0.5-0.7 at 23°C prior to imaging by fluorescence and differential interference contrast (DIC) microscopy. Representative images show DIC, Arx1-GFP localization, localization of a nucleus-specific RFP fusion protein, RFP-Pus1, and an image overlay (merge).

Supplementary Table 1. Yeast plasmids used in this study.

Plasmid	Description	Reference
pRS316	URA3 yeast centromeric vector	Sikorski and Hieter, 1989
pRS315	LEU2 yeast centromeric vector	Sikorski and Hieter, 1989
pRS316Zuo1	pRS316 carrying complete <i>ZUO1</i> gene under control of the native <i>ZUO1</i> promoter	Yan, 1998
pRS316Jjj1	pRS316 carrying complete <i>JJJ1</i> gene under control of the native <i>JJJ1</i> promoter	Meyer, 2007
pRS316Zuo1R247Aa	pRS316Zuo1 with R247 substituted to alanine	This study
pRS316Zuo1R251Aa	pRS316Zuo1 with R251 substituted to alanine	This study
pRS316Jjj1R221Ab	pRS316Jjj1 with R221 substituted to alanine	This study
pRS316Jjj1R225Ab	pRS316Jjj1 with R225 substituted to alanine	This study
pRS316Zuo1RR→AA	pRS316Zuo1 with R247 and R251 substituted to alanine	This study
pRS316Jjj1RR→AA	pRS316Jjj1 with R221 and R225 substituted to alanine	This study
pRS415GPDJjj1	pRS415 carrying complete <i>JJJ1</i> gene under control of the <i>GPD1</i> promoter	Meyer, 2007
pRS415GPDJjj1R221A	pRS415GPDJjj1 with R221 substituted to alanine	This study
pRS415GPDJjj1R225A	pRS415GPDJjj1 with R225 substituted to alanine	This study
pRS316Jjj1 1-339	pRS316Jjj1 with residues 340-590 deleted	This study
pRS316Jjj1 1-339RR→AA	pRS316Jjj1ΔC with R221 and R225 substituted to alanine	This study
pRS415GPDJjj1 1-339	pRS415GPDJjj1 with residues 340-590 deleted	This study
pRS315Jjj1	pRS315 carrying complete <i>JJJ1</i> gene under control of the native <i>JJJ1</i> promoter, cloned using BamHI and XbaI sites	This study
pRS315Jjj1 1-339	pRS315Jjj1 with residues 340-590 deleted	This study
pRS415CYCJjj1 1-339	pRS415 carrying <i>JJJ1</i> with codons for residues 340-590 deleted, under control of a weak (truncated) <i>CYC1</i> promoter	This study
pRS415ADHJjj1 1-339	pRS415 carrying <i>JJJ1</i> with codons for residues 340-590 deleted, under control of the <i>ADH1</i> promoter	This study
pRS316Jjj1 1-362	pRS316Jjj1 with residues 363-590 deleted	This study
pRS316Jjj1 1-389	pRS316Jjj1 with residues 390-590 deleted	This study
pRS316Jjj1 1-550	pRS316Jjj1 with residues 551-590 deleted	This study
pRS316Jjj1-GFP	pRS316Jjj1 carrying the <i>GFP</i> gene (<i>A. victoria</i>) cloned into the 3' BamHI site	This study
pRS316Jjj1 1-339-GFP	pRS316Jjj1-GFP with codons for Jjj1 residues 340-590 deleted	This study
pRS316Jjj1 1-362-GFP	pRS316Jjj1-GFP with codons for Jjj1 residues 363-590 deleted	This study
pRS316Jjj1 1-389-GFP	pRS316Jjj1-GFP with codons for Jjj1 residues 390-590 deleted	This study
pRS316Jjj1 1-550-GFP	pRS316Jjj1-GFP with codons for Jjj1 residues 551-590 deleted	This study
pRS313RFP-PUS1	pRS313 (<i>HIS3</i> yeast centromeric vector) carrying <i>RFP-PUS1</i> fusion gene	Han, 2007
pRS317RFP-PUS1	RFP-PUS1 cloned into pRS317 using SacI and Sall sites	This study

^a These plasmids are representative of other plasmids in Fig. 1B carrying single codon substitutions in *ZUO1* and were constructed in the same manner, in the same vector.

^b These plasmids are representative of other plasmids in Fig. 1C carrying single codon substitutions in *JJJ1* and were constructed in the same manner, in the same vector.

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