Supplemental table S1. Yeast strains used in this study

Name	Relevant genotype	Source
YAR1-TAP	*YAR1*-TAP::natNT2, *MAT* a	this study
RPS3-TAP	*RPS3*-TAP::natNT2, *MAT* a	this study
RPS2-TAP	*RPS2*-TAP::natNT2, *MAT* a	this study
KRR1-TAP	*KRR1*-TAP::natNT2, *MAT* a	this study
ENP1-TAP	*ENP1*-TAP::*TRP1* in MGD353-13D (*ade2, arg4, leu2, trp1, ura3*), *MAT* a	Cellzome
RIO2-TAP	*RIO2*-TAP::hisMX6, *MAT* a	this study
PWP2-TAP	*PWP2*-TAP::hisMX6, *MAT* a	this study
PWP2-TAP yar1Δ	*PWP2*-TAP::hisMX6, *yar1*::natNT2, *MAT* a	this study
UTP22-TAP	*UTP22*-TAP::natNT2, *MAT* a	this study
UTP22-TAP yar1Δ	*UTP22*-TAP::natNT2, *yar1*::hisMX6, *MAT* a	this study
YAR1-GFP	*YAR1*-GFP::natNT2, *ade3*::kanMX4, *MAT* α	this study
MNY8	*xpo1*::kanMX, pRS315-*crm1*-T539C in LLY1044 (*ade2, leu2, his3, trp1, ura3*), *MAT* a	(Neville and Rosbash, 1999)
MNY8 yar1Δ	*yar1*::natNT2 in MNY8	this study
yar1Δ	*yar1*::natNT2, *MATa*	this study
Rps3 shuffle	*rps3*::natNT2 *ade3* ::kanMX4 pRS316-*RPS3*-GFP, *MAT* α	this study
Rps3 shuffle yar1Δ	*rps3*::natNT2 *ade3* ::kanMX4 *yar1*::hisMX6 pRS316-*RPS3*-GFP, *MAT* α	this study
yar1-rps3 sl screening strain	*rps3*::natNT2 *ade3* ::kanMX4 *yar1*::hisMX6 pRS316-*RPS3*-GFP, pHT4467ΔCEN-*ADE3-HIS3-YAR1*, *MAT* α	this study
rps3Δ [RPS3]	*rps3*::natNT2, *ade3* ::kanMX4 pRS315-*RPS3*, *MAT* a	this study
rps3Δ [rps3-1]	*rps3*::natNT2, *ade3* ::kanMX4 pRS315-*rps3-1*, *MAT* a	this study
rps3Δ [rps3-11]	*rps3*::natNT2, *ade3* ::kanMX4 pRS315-*rps3-11*, *MAT* a	this study
rps3Δ yar1Δ [RPS3]	*rps3*::natNT2, *ade3* ::kanMX4 *yar1*::hisMX6 pRS315-*RPS3*, *MAT* a	this study
rps3Δ yar1Δ [rps3-11]	*rps3*::natNT2, *ade3* ::kanMX4 *yar1*::hisMX6 pRS315-*rps3-11*, *MAT* a	this study
NOP58-RedStar2 (Y3840)	*NOP58*-RedStar2::natNT2 in Ds1-2b (*leu2*, *his3*, *trp1*, *ura3*),*MAT* α	this study

All strains where no strain background is indicated are derived from W303 (*ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*).

Supplemental table S2. Yeast plasmids used in this study

Name	Relevant information	Source
pHT4467ΔCEN-*ADE3-HIS3*	CEN6 (instable), *HIS3*, *ADE3*	this study
pHT4467ΔCEN-*ADE3-HIS3-YAR1*	CEN6 (instable), *HIS3*, *ADE3, YAR1*	this study
pRS315-YAR1-GFP	CEN, *LEU2*	this study
pRS316-RPS3-GFP	CEN, *URA3*	(Yao et al., 2007)
pRS316-RPL25-GFP	CEN, *URA3*	(Gadal et al., 2001)
pADH111-RPS3(1-15)-(GA)5-3xyEGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xyeGFP	this study
pADH111-SV40NLS-(GA)5-3xyEGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xyeGFP	this study
pADH111-(GA)5-3xyEGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xyeGFP	this study
pRS315-YAR1	CEN, *LEU2*	this study
pRS315-RPS15	CEN, *LEU2*	this study
pRS315-RPS3	CEN, *LEU2*	this study
pRS315-rps3-1	CEN, *LEU2*, A136G, G191A and A404G nucleotide exchanges in *RPS3*	this study
pRS315-rps3-11 (E135K)	CEN, *LEU2*, A404G nucleotide exchange in *RPS3*	this study
pRS315-rps3-T46A	CEN, *LEU2*, A136G nucleotide exchange in *RPS3*	this study
pRS315-rps3-T46A/R64K	CEN, *LEU2*, A136G and G191A nucleotide exchanges in *RPS3*	this study
pRS315-rps3-R64K/E135K	CEN, *LEU2*, G191A and A404G nucleotide exchanges in *RPS3*	this study
pRS314-ADH1-YAR1	CEN, *TRP1*, P*ADH1*	this study
pCUP1-FLAG-Rps3	2µ, *URA3*, *leu2-d*, P*CUP1*	this study
pFA6a-kanMX6	for genomic deletion disruption	(Longtine et al., 1998)
pFA6a-natNT2	for genomic deletion disruption	(Janke et al., 2004)
pFA6a-hisMX6	for genomic deletion disruption	(Longtine et al., 1998)
pFA6a-GFP-natNT2	GFP(S65T), T*ADH1*, for C-terminal tagging	(Kressler et al., 2008)
pFA6a-RedStar2-natNT2	RedStar2, T*ADH1*, for C-terminal tagging	(Janke et al., 2004)
pFA6a-TAP-natNT2	TAP-tag, T*CYC1*, for C-terminal tagging	(Kressler et al., 2008)
pFA6a-TAP-HIS3MX6	TAP-tag, T*ADH1*, for C-terminal tagging	this study

P and T denote promoter and terminator, respectively. When no promoters and terminators are indicated, the authentic context was used.

Supplemental table S3. *E.coli* expression plasmids used in this study

Name	Relevant information	Source
pETDuet-1	AmpR, co-expression vector for *E.coli*	Novagen
pETDuet-YAR1	His6-tag fusion of *YAR1* in MCS1 of pETDuet-1	this study
pETDuet-YAR1-RPS3	His6-*YAR1* in MCS1 and FLAG-*RPS3* in MCS2 of pETDuet-1	this study
pETDuet-RPS3	His6-tag fusion of *RPS3* in MCS1 of pETDuet-1	this study
pETDuet-FLAG-RPS3	FLAG-RPS3 in MCS2 of pETDuet-1	this study

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1

(A) Synthetic lethality of *YAR1* and *RPS3*. *rps3Δ* and *rps3Δ yar1Δ* cells with pRS316-*RPS3* (*URA3*-selection marker) were transformed with plasmids that carry *RPS3* or *rps3-1* alleles. Cells were spotted in serial 10-fold dilution steps onto 5-FOA containing plates to counter-select against the pRS316 plasmid. As a growth control, strains were spotted onto SDC-leu plates. Plates were incubated at 30 °C for four days. Note that the *rps3Δ yar1Δ* strain carrying the *rps3-1* allele cannot lose the pRS316-*RPS3* plasmid and is therefore unable to grow on 5-FOA plates, indicating synthetic lethality. (B) An *RPS3*/*YAR1* shuffle strain was transformed with plasmids carrying the indicated wild-type (wt) and mutant *rps3* alleles. After 5-FOA shuffling, cells were spotted in 10-fold serial dilution steps onto YPD plates and incubated at the indicated temperatures for three days.

SUPPLEMENTAL REFERENCES

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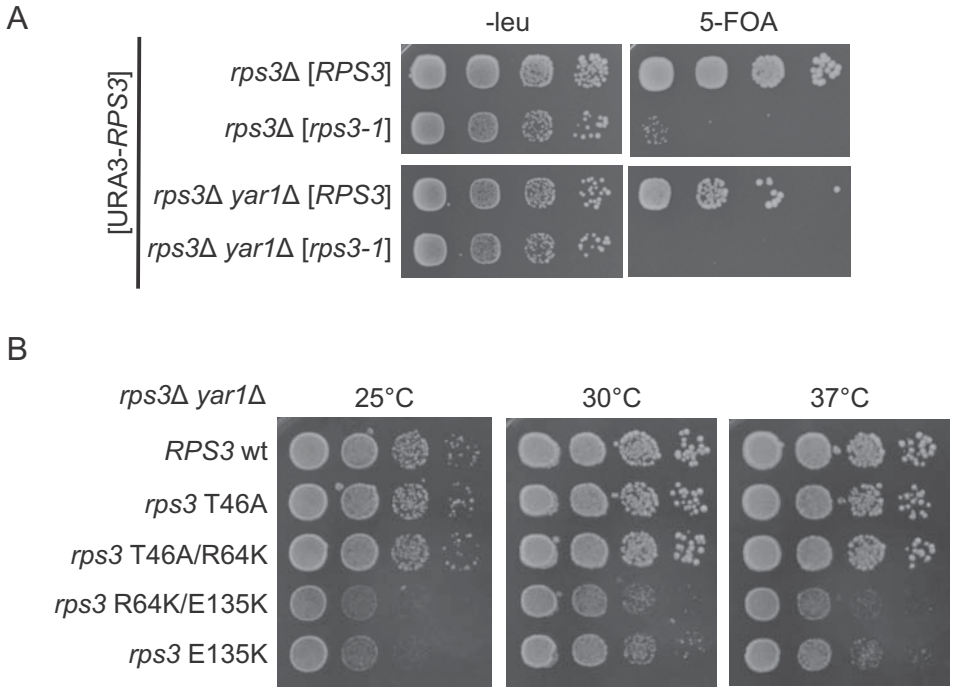
Figure S1


A
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5-FOA
[URA3-RPS3]
rps3Δ [RPS3]
rps3Δ [rps3-1]
rps3Δ yar1Δ [RPS3]
rps3Δ yar1Δ [rps3-1]
B
rps3Δ yar1Δ
25°C
30°C
37°C
RPS3 wt
rps3 T46A
rps3 T46A/R64K
rps3 R64K/E135K
rps3 E135K