

**Supplemental table S1. Yeast strains used in this study**

Name	Relevant genotype	Source
YAR1-TAP	<i>YAR1-TAP::natNT2, MAT a</i>	this study
RPS3-TAP	<i>RPS3-TAP::natNT2, MAT a</i>	this study
RPS2-TAP	<i>RPS2-TAP::natNT2, MAT a</i>	this study
KRR1-TAP	<i>KRR1-TAP::natNT2, MAT a</i>	this study
ENP1-TAP	<i>ENP1-TAP::TRP1</i> in MGD353-13D ( <i>ade2, arg4, leu2, trp1, ura3</i> ), <i>MAT a</i>	Cellzome
RIO2-TAP	<i>RIO2-TAP::hisMX6, MAT a</i>	this study
PWP2-TAP	<i>PWP2-TAP::hisMX6, MAT a</i>	this study
PWP2-TAP <i>yar1Δ</i>	<i>PWP2-TAP::hisMX6, yar1::natNT2, MAT a</i>	this study
UTP22-TAP	<i>UTP22-TAP::natNT2, MAT a</i>	this study
UTP22-TAP <i>yar1Δ</i>	<i>UTP22-TAP::natNT2, yar1::hisMX6, MAT a</i>	this study
YAR1-GFP	<i>YAR1-GFP::natNT2, ade3::kanMX4, MAT α</i>	this study
MNY8	<i>xpo1::kanMX, pRS315-crm1-T539C</i> in LLY1044 ( <i>ade2, leu2, his3, trp1, ura3</i> ), <i>MAT a</i>	(Neville and Rosbash, 1999)
MNY8 <i>yar1Δ</i>	<i>yar1::natNT2</i> in MNY8	this study
<i>yar1Δ</i>	<i>yar1::natNT2, MAT a</i>	this study
Rps3 shuffle	<i>rps3::natNT2 ade3::kanMX4 pRS316-RPS3-GFP, MAT α</i>	this study
Rps3 shuffle <i>yar1Δ</i>	<i>rps3::natNT2 ade3::kanMX4 yar1::hisMX6 pRS316-RPS3-GFP, MAT α</i>	this study
<i>yar1-rps3</i> sl screening strain	<i>rps3::natNT2 ade3::kanMX4 yar1::hisMX6 pRS316-RPS3-GFP, pHT4467ΔCEN-ADE3-HIS3-YAR1, MAT α</i>	this study
<i>rps3Δ</i> [RPS3]	<i>rps3::natNT2, ade3::kanMX4 pRS315-RPS3, MAT a</i>	this study
<i>rps3Δ</i> [ <i>rps3-1</i> ]	<i>rps3::natNT2, ade3::kanMX4 pRS315-rps3-1, MAT a</i>	this study
<i>rps3Δ</i> [ <i>rps3-11</i> ]	<i>rps3::natNT2, ade3::kanMX4 pRS315-rps3-11, MAT a</i>	this study
<i>rps3Δ yar1Δ</i> [RPS3]	<i>rps3::natNT2, ade3::kanMX4 yar1::hisMX6 pRS315-RPS3, MAT a</i>	this study
<i>rps3Δ yar1Δ</i> [ <i>rps3-11</i> ]	<i>rps3::natNT2, ade3::kanMX4 yar1::hisMX6 pRS315-rps3-11, MAT a</i>	this study
NOP58-RedStar2 (Y3840)	<i>NOP58-RedStar2::natNT2</i> in Ds1-2b ( <i>leu2, his3, trp1, ura3</i> ), <i>MAT α</i>	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), <i>HIS3</i> , <i>ADE3</i>	this study
pHT4467ΔCEN-ADE3-HIS3-YAR1	CEN6 (instable), <i>HIS3</i> , <i>ADE3</i> , <i>YAR1</i>	this study
pRS315-YAR1-GFP	CEN, <i>LEU2</i>	this study
pRS316-RPS3-GFP	CEN, <i>URA3</i>	(Yao et al., 2007)
pRS316-RPL25-GFP	CEN, <i>URA3</i>	(Gadal et al., 2001)
pADH111-RPS3(1-15)-(GA)5-3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal 3xyeGFP	this study
pADH111-SV40NLS-(GA)5-3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal 3xyeGFP	this study
pADH111-(GA)5-3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal 3xyeGFP	this study
pRS315-YAR1	CEN, <i>LEU2</i>	this study
pRS315-RPS15	CEN, <i>LEU2</i>	this study
pRS315-RPS3	CEN, <i>LEU2</i>	this study
pRS315-rps3-1	CEN, <i>LEU2</i> , A136G, G191A and A404G nucleotide exchanges in <i>RPS3</i>	this study
pRS315-rps3-11 (E135K)	CEN, <i>LEU2</i> , A404G nucleotide exchange in <i>RPS3</i>	this study
pRS315-rps3-T46A	CEN, <i>LEU2</i> , A136G nucleotide exchange in <i>RPS3</i>	this study
pRS315-rps3-T46A/R64K	CEN, <i>LEU2</i> , A136G and G191A nucleotide exchanges in <i>RPS3</i>	this study
pRS315-rps3-R64K/E135K	CEN, <i>LEU2</i> , G191A and A404G nucleotide exchanges in <i>RPS3</i>	this study
pRS314-ADH1-YAR1	CEN, <i>TRP1</i> , <i>PADH1</i>	this study
pCUP1-FLAG-Rps3	2μ, <i>URA3</i> , <i>leu2-d</i> , <i>PCUP1</i>	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), <i>TADH1</i> , for C-terminal tagging	(Kressler et al., 2008)
pFA6a-RedStar2-natNT2	RedStar2, <i>TADH1</i> , for C-terminal tagging	(Janke et al., 2004)
pFA6a-TAP-natNT2	TAP-tag, <i>TCYCI</i> , for C-terminal tagging	(Kressler et al., 2008)
pFA6a-TAP-HIS3MX6	TAP-tag, <i>TADH1</i> , 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 <i>E.coli</i>	Novagen
pETDuet-YAR1	His6-tag fusion of <i>YAR1</i> in MCS1 of pETDuet-1	this study
pETDuet-YAR1-RPS3	His6- <i>YAR1</i> in MCS1 and FLAG- <i>RPS3</i> in MCS2 of pETDuet-1	this study
pETDuet-RPS3	His6-tag fusion of <i>RPS3</i> in MCS1 of pETDuet-1	this study
pETDuet-FLAG-RPS3	FLAG- <i>RPS3</i> 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

