

Supplementary Information

Nuclear import of dimerized ribosomal protein Rps3 in complex with its chaperone

Yar1

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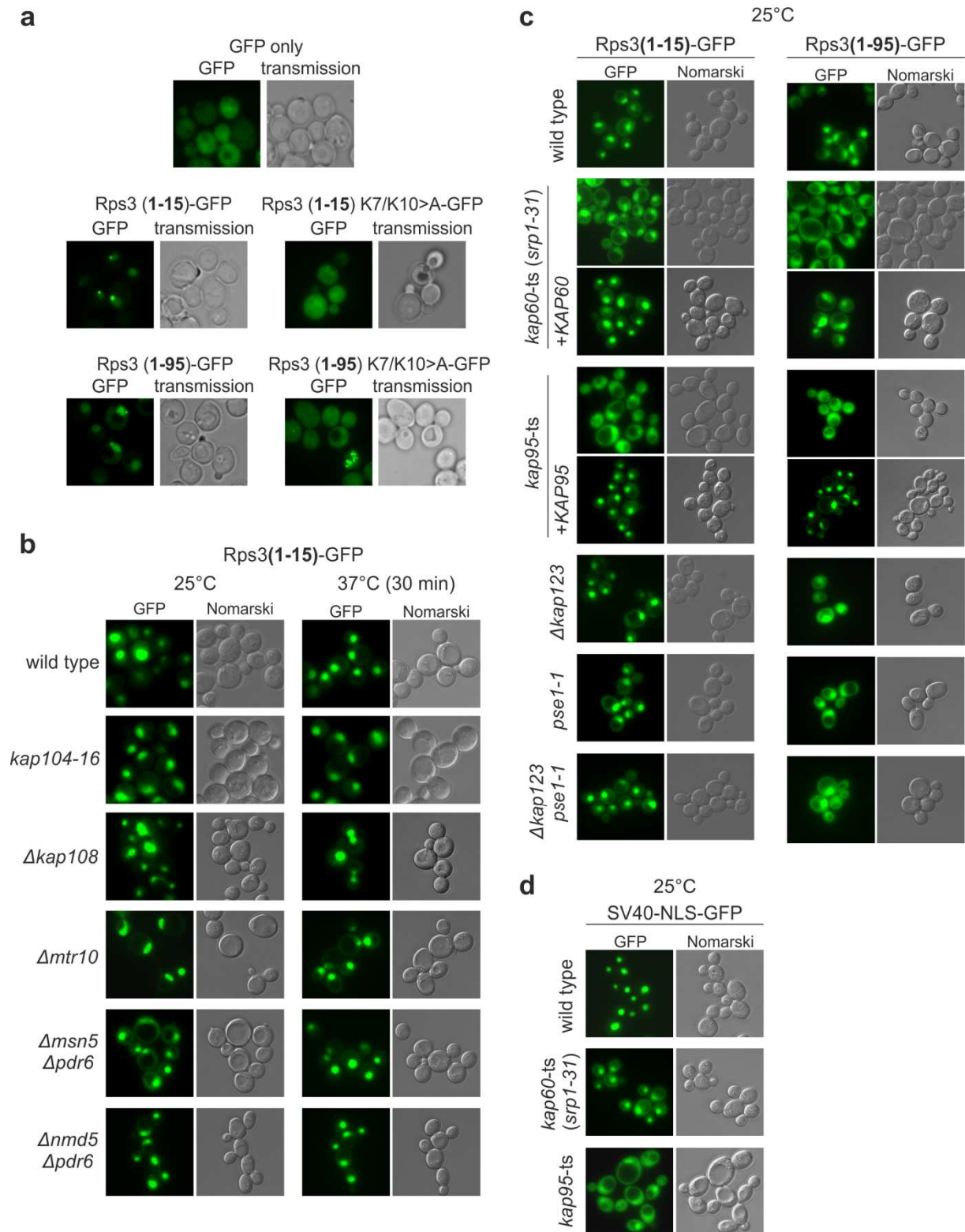
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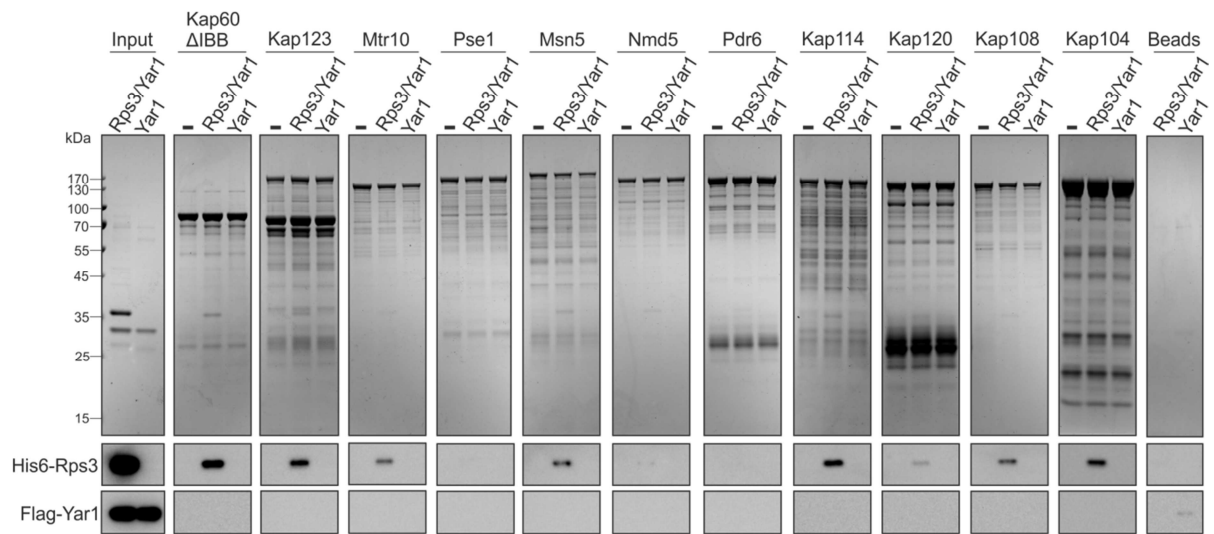
Supplementary Figure 1



Supplementary Figure S1. Kap60/Kap95, Kap123 and Pse1 are required for efficient nuclear import of Rps3. The localization of the indicated N-terminal Rps3 reporter constructs (a), (b) and (c) or SV-40NLS (d) fused to 3xyEGFP was assessed by

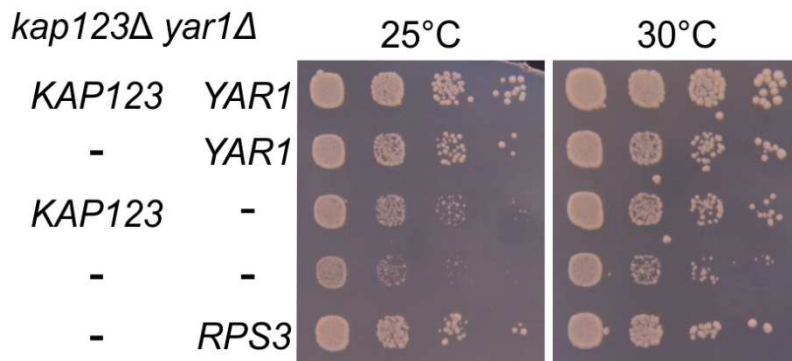
fluorescence microscopy in W303 wild-type or the indicated karyopherin mutant strains after incubation at the indicated temperatures. (a) 3xyEGFP alone was used as control and displayed predominantly cytoplasmic localization.

Supplementary Figure 2



Supplementary Figure S2. Rps3 is transferred from the Rps3/Yar1 complex onto importins. The indicated GST-tagged importins were expressed in *E. coli*, immobilized on glutathione-agarose beads and subsequently incubated with purified His6-Rps3/Flag-Yar1 complex, purified Flag-Yar1 or buffer (-). As negative control, empty glutathione-agarose beads were incubated with the His6-Rps3/Flag-Yar1 complex or Flag-Yar1 (beads). After subsequent washing steps, bound material was eluted and analyzed by SDS-PAGE and Coomassie staining or Western blotting with the indicated antibodies. Notably, Yar1 was not detected bound to any of the tested importins, also not in the samples where Rps3-binding was observed.

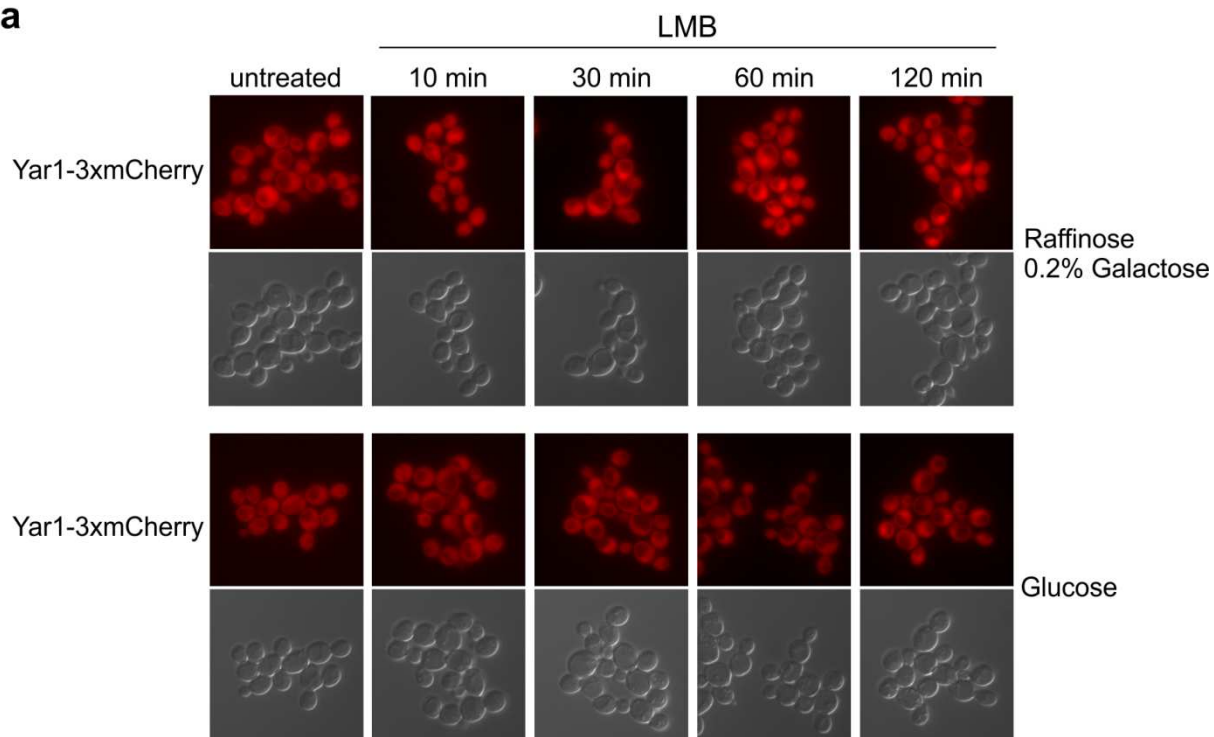
Supplementary Figure 3



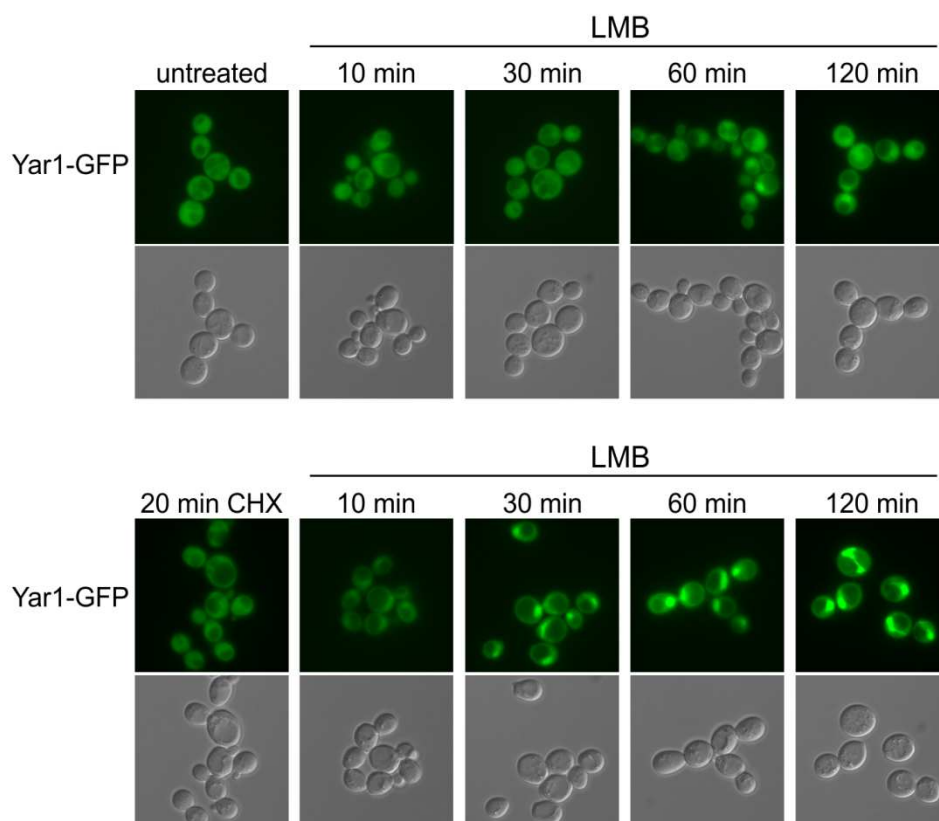
Supplementary Figure S3. Deletion of *KAP123* slightly enhances the growth defect of a *yar1* deletion strain. A *kap123Δ yar1Δ* strain was transformed with plasmids harboring the indicated wild-type alleles or empty plasmids (-). Cells were spotted in 10-fold serial dilutions on SD-Ura-Leu plates and incubated at the indicated temperatures for 3 days.

Supplementary Figure 4

a

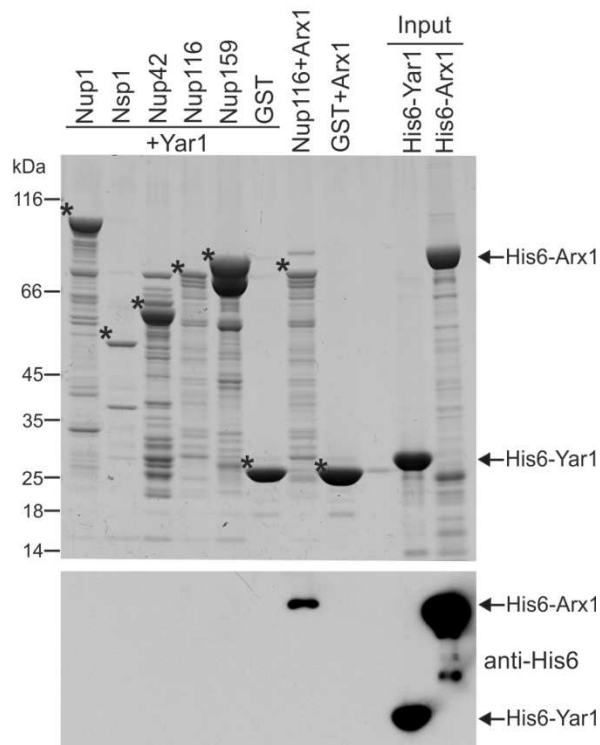


b



Supplementary Figure S4. Yar1 enters the nucleus after Rps3 depletion or inhibition of protein synthesis. (a) A 3xmCherry tag was C-terminally fused to the *YAR1* gene locus in a leptomycin B (LMB)-sensitive *crm1T539C rps3Δ* mutant strain in which plasmid-encoded *RPS3* was expressed under the control of a *GAL1* promoter. The localization of the Yar1-3xmCherry fusion protein was assessed under conditions allowing Rps3 expression (medium containing 2% raffinose and 0.2% galactose as carbon source) (upper panel) or after depletion of Rps3 for 2h in medium containing glucose as carbon source (lower panel). The Yar1-3xmCherry localization was examined in untreated cells or after addition of LMB for the indicated time. **(b)** A LMB-sensitive *crm1T539C yar1Δ* strain was transformed with a plasmid encoding a Yar1-eGFP fusion protein. The localization of Yar1-eGFP was assessed in untreated cells or in cells treated with LMB for the indicated time (upper panel). The localization of Yar1-eGFP was assessed after incubation for 20 min with cycloheximide (CHX) prior to LMB addition (lower panel).

Supplementary Figure 5



Supplementary Figure S5. Yar1 does not interact with nucleoporins. The indicated GST-tagged nucleoporins (or truncations thereof; see Table S3) were immobilized on glutathione-agarose beads and incubated with purified His6-Yar1. As positive control the Nup116 FG-repeat fragment was incubated with purified His6-Arx1 (pre-60S export factor). Bound material was eluted in buffer containing 20 mM reduced glutathione and samples were subsequently analyzed by SDS-PAGE and Coomassie staining or Western blotting. Asterisks indicate the respective bait proteins.

Table S1. *S. cerevisiae* strains

Name	Genotype	Source
W303	<i>ade2-1, his3-11, 15, leu2-3,112, trp1-1, ura3-1, can1-100</i>	¹
<i>srp1-31/kap60ts</i>	W303 MATa <i>srp1-31</i>	²
<i>kap95ts</i>	MATa <i>leu2 his3 trp1 ura3 rsl1-4</i>	Ed Hurt lab, backcross from PSY1103 ³ with W303
Δ <i>kap123</i> (PSY967)	W303 MATa <i>kap123::HIS3</i>	⁴
<i>pse1-1</i> (PSY1201)	W303 MATa <i>pse1-1</i>	⁴
Δ <i>kap123 pse1-1</i> (PSY1042)	W303 MATa <i>pse1-1 kap123::HIS3</i>	⁴
Δ <i>sxm1</i> (PSY1200)	W303 MATa <i>sxm1::HIS3</i>	⁴
Δ <i>mtr10</i>	W303 MATa <i>mtr10::HIS3</i>	⁵
Δ <i>msn5 \Delta</i> <i>pdr6</i>	W303 MATa <i>pdr6::HIS3 msn5::TRP1</i>	Ed Hurt lab
Δ <i>nmd5 \Delta</i> <i>pdr6</i>	W303 MATa <i>pdr6::HIS3 nmd5::HIS3</i>	Ed Hurt lab
RPS3-TAP KAP60-3xHA	W303 MATa RPS3-TAP::natNT2 KAP60-3xHA:: HIS3MX6	this study
YAR1-TAP KAP60-3xHA	W303 MATa YAR1-TAP::natNT2 KAP60-3xHA:: HIS3MX6	this study
KAP60-3xHA	W303 MATa KAP60-3xHA:: HIS3MX6	this study
YAR1-TAP RPS3-Flag	W303 MATa YAR1-TAP::HIS3MX6 RPS3-Flag::natNT2	this study
<i>srp1-31 \Delta</i> <i>yar1</i>	W303 MATa <i>srp1-31 yar1::HIS3MX6</i>	this study (<i>YAR1</i> knockout in <i>srp1-31</i>)
<i>kap95ts \Delta</i> <i>yar1</i>	MATa <i>leu2 his3 trp1 ura3 rsl1-4 yar1::HIS3MX6</i>	this study (<i>YAR1</i> knockout in <i>kap95ts</i>)
KAP104 shuffle	MATa <i>kap104::natNT2 ade3::kanMX4 [pRS316-KAP104]</i>	⁶
Δ <i>kap123 \Delta</i> <i>yar1</i>	W303 MATa <i>ade3::kanMX4 yar1::natNT2 kap123::HIS3MX6</i>	this study
<i>crm1T539C YAR1-3xmCherry pGAL111-RPS3</i>	W303 MATa <i>ADE2 crm1T539C::kanMX4 YAR1-3xmCherry::hphNT1 rps3::natNT2 [pGAL111-RPS3]</i>	this study
Δ <i>crm1 \Delta</i> <i>yar1 pRS315-crm1T539C</i>	W303 MATa <i>crm1::kanMX yar1::natNT2 [pRS315-crm1T539C]</i>	this study (based on MNY8 strain from Neville and Rosbash ⁷)

Table S2. *S. cerevisiae* plasmids

Name	Relevant Information	Source
pADH111-RPS3(1-15)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	⁸
pADH111-RPS3(1-15.KKRK>A)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	this study
pADH111-RPS3(1-15.K7/K10>A)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	this study
pADH111-RPS3(1-95)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	this study
pADH111-RPS3(1-95.KKRK>A)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	this study
pADH111-RPS3(1-95.K7/K10>A)-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	this study
pADH111- (GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	⁸
pADH111-SV40-NLS-(GA) ₅ -3xyEGFP	CEN, <i>LEU2</i> , <i>PADH1</i> , <i>TADH1</i> , C-terminal (GA) ₅ 3xyEGFP	⁸
pRS314-KAP60	CEN, <i>URA3</i>	⁹
pRS314-KAP95	CEN, <i>URA3</i>	Ed Hurt lab, subcloned from ¹⁰
pRS315-YAR1	CEN, <i>LEU2</i> , <i>PYAR1</i> , <i>TYAR1</i>	⁸
pRS315-RPS3	CEN, <i>LEU2</i> , <i>PRPS3</i> , <i>TRPS3</i>	⁸
pRS314-kap104-16	CEN, <i>TRP1</i> , <i>PKAP104</i> , <i>TKAP104</i>	¹¹
YCplac22-KAP123	CEN, <i>TRP1</i> , <i>PKAP123</i> , <i>TKAP123</i>	this study
pGAL111-RPS3	CEN, <i>LEU2</i> , <i>PGAL1</i> , <i>TADH1</i>	this study
pRS316-YAR1-EGFP	CEN, <i>URA3</i> , <i>PYAR1</i> , <i>TADH1</i>	⁸

P and T denote promoter and terminator, respectively.

Table S3. *E. coli* expression plasmids

Name	Relevant Information	Source
pETDuet-1-His6-Rps3/Flag-Yar1	amp ^r , T7 promoter/ <i>lac</i> operator	this study
pETDuet-1-His6-Rps3(K7/K10>A)/Flag-Yar1	amp ^r , T7 promoter/ <i>lac</i> operator	this study
pETDuet-1-Flag-Yar1	amp ^r , T7 promoter/ <i>lac</i> operator	¹²
pETDuet-1-His6-Yar1	amp ^r , T7 promoter/ <i>lac</i> operator	⁸
pProEx-GST-TEV-Kap60ΔIBB (Kap60 amino acids 81-542)	amp ^r , TRC promoter/ <i>lac</i> operator	this study
pGEX-4TEV-KAP123	amp ^r , TAC promoter/ <i>lac</i> operator	¹³
pGEX-4TEV-PSE1	amp ^r , TAC promoter/ <i>lac</i> operator	¹³
pGEX-4TEV-KAP104	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁴
pGEX-4T-SXM1	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-4T-NMD5	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-5G-KAP120	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-4T-KAP114	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-4T-PDR6	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-4TEV-MTR10	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-4T-MSN5	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁵
pGEX-Nup1 (amino acids 332-1076)	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁶
pGEX-Nsp1-C (amino acids 591-823)	kan ^r , TAC promoter/ <i>lac</i> operator	¹⁷
pGEX-Nup42	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁶
pGEX-Nup116 (amino acids 165-715)	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁶
pGEX-Nup159 (amino acids 457-900)	amp ^r , TAC promoter/ <i>lac</i> operator	¹⁸
pET32a-Arx1	amp ^r , T7 promoter/ <i>lac</i> operator	this study

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