Timney et al., http://www.jcb.org/cgi/content/full/jcb.200608141/DC1

Supplemental materials and methods

Identification of Kap123p-binding proteins by overlay assay

Kap123p–cargo interactions were identified in HPLC separations of yeast nuclear proteins (Rout and Blobel, 1993; Rout et al., 1997) by overlay assays, exactly as we described for the identification of Kap121p cargoes (Leslie et al., 2002, 2004). Kap123p recognized a distinct pattern of proteins, as compared with those recognized by the karyopherins Kap104p, Kap121p, and Kap95p/Kap60p (Fig. S1). Comparison of the profiles of molecular weights of proteins recognized by these four transport factors showed that Kap123p prefers proteins with molecular weights 20 amino acids, rich in lysine and arginine, with few acidic residues, and frequently terminated at one or both ends by prolines or glycines. If these sequences caused a nuclear accumulation of GFP fusion proteins that was sensitive to the transport poison mixture (see import assay), they were defined as putative NLSs and taken for further study. In all five cases, such NLSs were defined (Table I and Fig. S3). Indeed, independent research on YRA1 confirmed the region selected by these criteria to be its single NLS (Zenklusen et al., 2001).

As cargoes can share transport pathways (Leslie et al., 2004), we tested whether import of each fusion protein was dependent on Kap123p (Fig. S3). The test chosen was whether the N/C ratio of each NLS-GFP was significantly altered in Δ*kap123* yeast compared with the wild type strain. Control experiments showed no significant decrease in N/C ratio for known non-Kap123p cargoes in Δ*kap123* yeast (e.g., Pho4NLS-GFP).

As previously stated (Figs. 2 and 3), both ribosomal NLS fusion proteins (Rpl25NLS-GFPp and Rps1bNLS-GFPp) proved to be profoundly mislocalized in the absence of Kap123p. In contrast, three of the four nonribosomal NLS-GFP cargoes were mislocalized to lesser (although highly statistically significant) extents in Δ*kap123* yeast (Fig. 3).

Plasmid construction

All plasmids used in this study are briefly described with details of restriction sites used in Table S1. Plasmids were constructed by standard recombinant DNA methods (Sambrook and Russell, 2001). Genes were amplified from *S. cerevisiae* genomic DNA by PCR, using primers encoding appropriate restriction sites. PCR products were trapped in TA Cloning vector (Invitrogen) and subcloned into the appropriate plasmid for expression in yeast or bacteria.

The bacterial expression plasmid encoding the GST-GFP fusion protein was a gift from J. Rosenblum (ActivX BioSciences, Inc., La Jolla, CA).

NLSs were used as previously defined for *RPL25* (Schaap et al., 1991) and *NAB2* (Lee and Aitchison, 1999). Observation of the sequence surrounding the proposed NLS of *PHO4* (Kaffman et al., 1998) indicated that the full extent of the NLS might be greater than previously described. Indeed, inclusion of a lysine-rich sequence downstream of the initially described NLS (Table I) significantly increased the nuclear accumulation of *PHO4NLS-GFP* well beyond that expected by simply increasing cargo size. The distribution of the expanded Pho4NLS fusion protein continued to be fully dependent on Kap121p

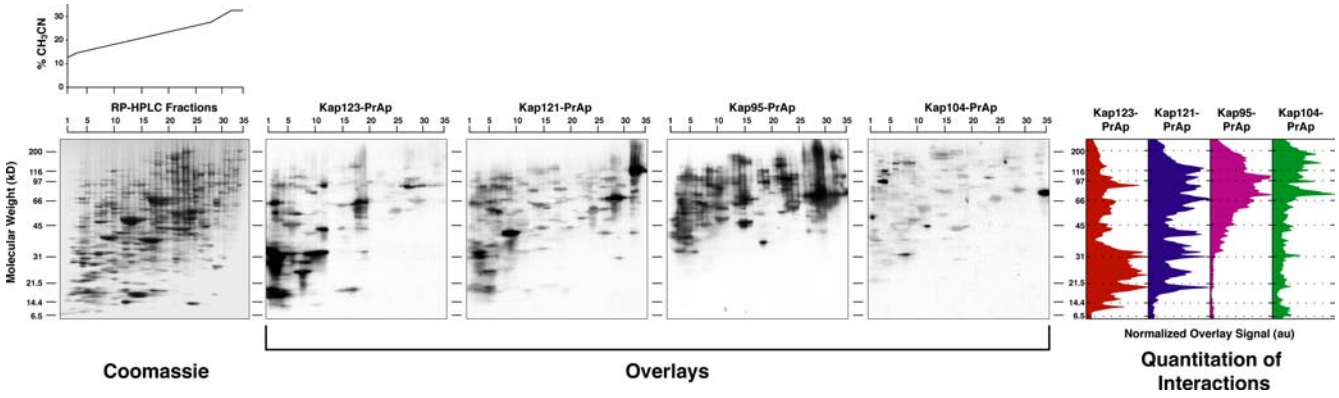

Figure S1. **Kap123p interacts with a distinct subset of small nuclear proteins.** (left) Yeast nucleoplasmic proteins were resolved by reverse-phase HPLC using a gradient of acetonitrile with the profile illustrated in the top left graph. Resulting fractions were separated by SDS-PAGE and stained with Coomassie blue. (middle) Proteins from unstained gels were transferred to nitrocellulose and probed in an overlay assay with cytosol from the indicated KAP-PrA strains. Kap-PrA binding was detected using chemiluminescent detection of the PrA tag. (right) The intensity of binding down the entire molecular weight range of each overlay blot was summed for all fractions, divided by the corresponding Coomassie staining profile, and normalized to a maximum value of 1.

Table S1. **Plasmids**

Descriptive name	Formal name	Construction	Source
pYX242	pYX242	2μ yeast constitutive expression plasmid (TPI1 promotor)	Novagen/R&D Systems
eGFP	pEGFP3	eGFP ORF inserted downstream of HindIII in pYX242	J. Aitchison
RPL25NLS-GFP	pBT008	NLS of RPL25 (bp 1–135) fused upstream of eGFP in EcoRI–SalI of pYX242	This study
RPS1BNLS-GFP	pBT013	NLS of RPS1B (bp 1–150) inserted upstream of eGFP in EcoRI–HindIII of pEGFP3	This study
MAK16NLS-GFP	pBT015	NLS of MAK16 (bp 799–861) fused upstream of eGFP in BamHI–SalI of pYX242	This study
NUG1NLS-GFP	pBT017	NLS of NUG1 (bp 1–183) inserted upstream of eGFP in EcoRI–HindIII of pEGFP3	This study
YIL096CNLS-GFP	pBT022	NLS of YIL096C (bp 1–202) inserted upstream of eGFP in BamHI–HindIII of pEGFP3	This study
YRA1NLS-GFP	pBT023	NLS of YRA1 (bp 40–210) inserted upstream of eGFP in EcoRI–HindIII of pEGFP3	This study
RPL25NLS-GFP-PRA	pBT009	NLS of RPL25 (bp 1–135) fused upstream of eGFP in EcoRI–HindII of pYX242, followed by a single PrA repeat in HindIII–SalI	This study
RPL25NLS-YFP	pBT011	NLS of RPL25 (bp 1-120) fused upstream of eYFP in EcoRI–SalI of pYX242	This study
NAB2NLS-GFP-PRA	pBT016	NLS of NAB2 (bp 601–750) inserted in EcoRI–BamHI, eGFP inserted downstream in BamHI–HindIII, and a single PrA repeat inserted further downstream in HindIII–SalI of pYX242	This study
PHO4NLS-GFP-PRA	pBT018	NLS of PHO4 (bp 421–588) inserted in EcoRI–BamHI, eGFP inserted downstream in BamHI–HindIII, and a single PrA repeat inserted further downstream in HindIII–SalI of pYX242	This study
PHO4NLS-YFP	pBT031	NLS of PHO4 (bp 421–588) inserted in EcoRI–HindIII and eYFP inserted downstream in HindIII–SalI of pYX242	This study
NAB2NLS-GFP	pBT032	NLS of NAB2 (bp 601–750) inserted upstream of eGFP in EcoRI–HindIII of pEGFP3	This study
rpl25NLS-GFP (K21A K22A)	pBT012	K21A and K22A mutations introduced into RPL25NLS in pBT008 by site-directed mutagenesis	This study
pYEX-BX	pYEX-BX	2μ yeast copper-inducible (CUP1 promotor) expression plasmid	CLONTECH Laboratories, Inc.
pYEX-BX (leu2 URA3)	pBT024	Early frameshift mutation introduced into LEU2 of pYEX-BX by cutting with BstEII, blunt ending, and religating the vector back together	This study
KAP95-HA	pBT025	KAP95 fused upstream of two HA tags in BamHI–SalI of pBT024	This study
KAP104-HA	pBT026	KAP104 fused upstream of two HA tags in BamHI–SalI of pBT024	This study
KAP121-HA	pBT027	KAP121 fused upstream of two HA tags in BamHI–SalI of pBT024	This study
KAP123-HA	pBT028	KAP123 fused upstream of two HA tags in SalI of pBT024	This study
KAP121-CFP	pBT029	KAP121 inserted in BamHI–SalI of pBT024; CFP was then inserted in MluI–AvrII encoded by the KAP121 3′ PCR primer	This study
KAP123-CFP	pBT030	KAP123 inserted in SalI of pBT024; CFP was then inserted in MluI–AvrII encoded by the KAP121 3′ PCR primer	This study
RPL25NLS-GFP-HIS	pJN001	RPL25NLS-GFP amplified from pBT001, with AseI and SalI sites engineered into upstream and downstream PCR primers respectively; fragment inserted into NdeI–SalI of pET21b	This study
rpl25NLS-GFP-HIS (K21A K22A)	pJN002	Mutated rpl25NLS-GFP amplified from pBT009, with AseI and SalI sites engineered into upstream and downstream PCR primers respectively; fragment inserted into NdeI–SalI of pET21b	This study
PHO4NLS-YFP-HIS	pJN003	PHO4NLS-YFP from pBT031 in NdeI–SalI of pET21b	This study
NAB2NLS-GFP-HIS	pJN004	NAB2NLS-GFP amplified from pBT032, with AseI and SalI sites engineered into upstream and downstream PCR primers respectively; fragment inserted into NdeI–SalI of pET21b	This study
GST-GFP	GST-GFP	GFP ORF in SalI–NotI of pGEX-4T3	J. Rosenblum
GST-KAP104	GST-KAP104	KAP104 in BamHI of pGEX-2TK	Lee and Aitchison, 1999
GST-KAP121	GST-KAP121	KAP121 in BamHI–XhoI of pGEX-4T1	Marelli et al., 1998
GST-KAP123	pJN005	KAP123-HA from pBT028 in SalI of pGEX-4T3	This study

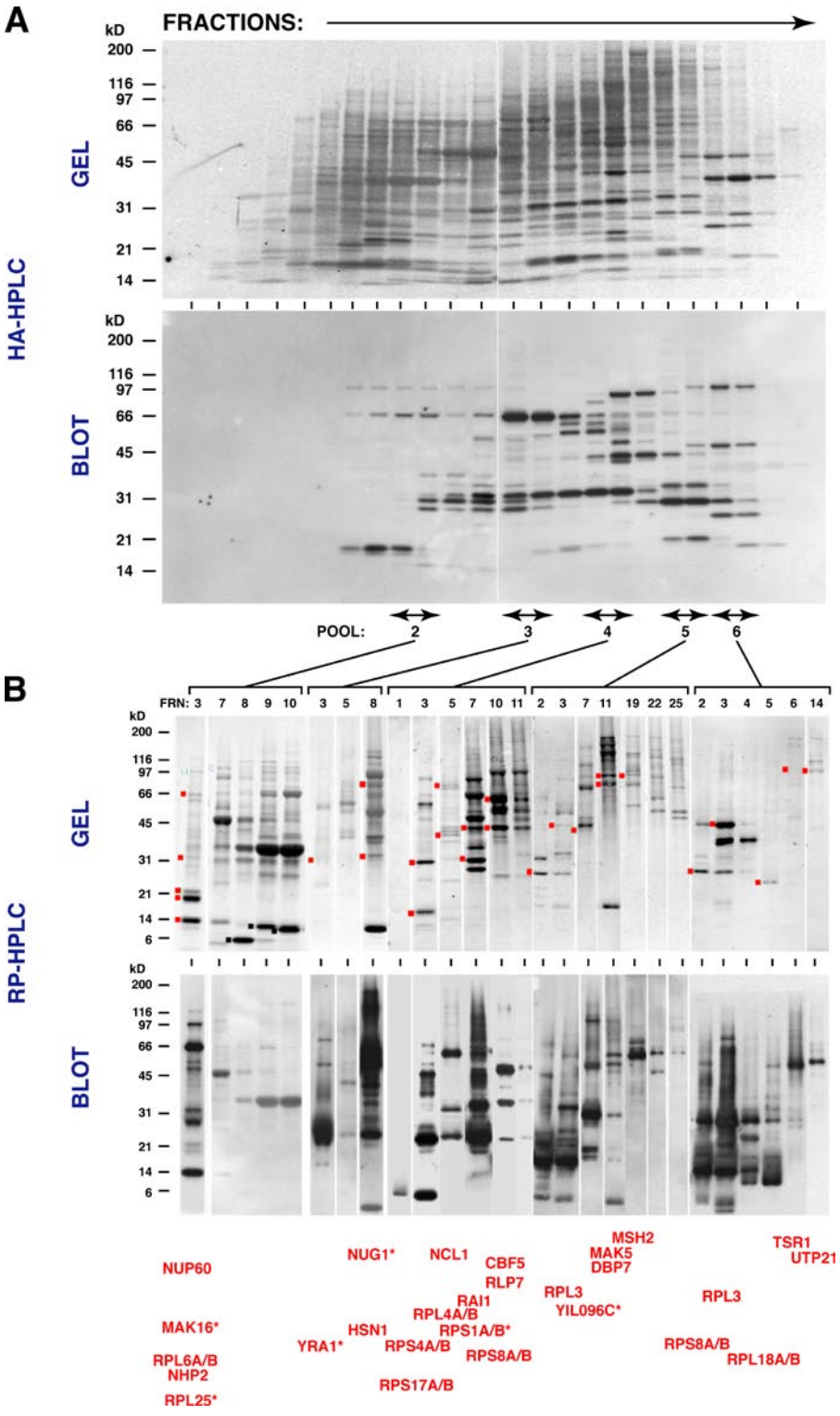

Figure S2. **The identities of Kap123p-interacting proteins were determined from overlay assays.** (A) Yeast nucleoplasm was fractionated by hydroxylapatite-HPLC, the fractions were separated by SDS-PAGE, and the resulting gels were transferred to nitrocellulose and stained with Amido black (top) before being probed for binding to Kap123-PrAp by overlay assay as before (bottom). (B) Fractions of the HA-HPLC separation that indicated a large number of interactions with Kap123p were pooled. Each pool was then fractionated further by reverse-phase HPLC and, finally, separated by SDS-PAGE. Overlay assays were once again performed from these blots to determine which protein bands bound strongly to Kap123p (red squares). The identities of these Kap123p-interacting proteins were determined by mass spectrometry of the corresponding bands (bottom). Those proteins that were selected for further investigation are marked with asterisks.

for its import (experiments in *kap121* temperature-sensitive yeast). The primary sequences of each of the NLSs used are given in Table I.

All NLS-GFP yeast expression constructs were made in the pYX242 backbone (Novagen; discontinued) that constitutively expresses genes at a high level under the *TPI1* promotor. The smaller NLSs of *RPL25* and *MAK16* were encoded into PCR primers for eGFP, but the larger NLS sequences of *RPS1B*, *NAB2*, *PHO4*, *MAK16*, *NUG1*, *YIL096c*, and *YRA1* were cloned by PCR and subcloned into appropriate pYX242 derivatives. *RPL25NLS*, *NAB2NLS*, and *PHO4NLS* were cloned as NLS-GFP constructs that optionally had an additional 174-bp repeat of PrA added to their C termini. *RPL25NLS* was also cloned as a fusion protein to eYFP (CLONTECH Laboratories, Inc.), which was otherwise identical to the eGFP version.

Table S2. **Strains**

Description	Formal name	Genotype	Source
WT	DF5a	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*	Finley et al., 1987
Δ*kap123* (URA3)	MRY001	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal kap123::URA3*	Rout et al., 1997
Δ*kap123* (TRP1)	BTY001	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1*	*TRP1* marker swap for *URA3* of MRY001
KAP104-GFP	KAP104-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KAP104-GFP::HIS3MX6*	Invitrogen; Huh et al., 2003
KAP121-GFP	KAP121-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KAP121-GFP::HIS3MX6*	Invitrogen; Huh et al., 2003
KAP123-GFP	KAP123-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KAP123-GFP::HIS3MX6*	Invitrogen; Huh et al., 2003
KAP95-GFP	KAP95-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KAP95-GFP::HIS3MX6*	Invitrogen; Huh et al., 2003
KAP95-PRA	MRY002	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal KAP95-PRA::HIS3::URA3*	Rout et al., 1997
KAP104-PRA	MRY003	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal KAP104-PRA::HIS3::URA3*	Aitchison et al., 1996
KAP121-PRA	MRY004	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal KAP121-PRA::HIS3::URA3*	Rout et al., 1997
KAP123-PRA	MRY005	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal KAP123-PRA::HIS3::URA3*	Rout et al., 1997
HTB2-CFP	BTY002	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal HTB2-CFP::HIS3MX*	*HTB2* C- terminus tagged with CFP in DF5
TPI1-CFP	BTY003	*MATα trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX*	*TPI1* C terminus tagged with CFP in DF5
WT-Import a	BTY004	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX HTB2-CFP::HIS3MX*	Segregant of tetrad resulting from mating of BTY002 and BTY003
WT-Import α	BTY005	*MATα trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX HTB2-CFP::HIS3MX*	Segregant of tetrad resulting from mating of BTY002 and BTY003
Δ*kap123*-Import a	BTY006	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal HTB2-CFP::HIS3MX TPI1-CFP::HIS3MX kap123::ura3::TRP1*	Segregant of tetrad resulting from mating of BTY005 and BTY001
RPL25NLS-GFP Δ*kap123*	BTY009	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1* pBT008 *(LEU2)*	BTY001 transformed with pBT008
rpl25nls-GFP (K21A K22A) WT	BTY010	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* pBT012 *(LEU2)*	DF5a transformed with pBT012
RPL25NLS-GFP WT	BTY011	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* pBT008 *(LEU2)*	DF5a transformed with pBT008
RPL25NLS-GFP-PRA Δ*kap123*	BTY012	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1* pBT009 *(LEU2)*	BTY001 transformed with pBT009
RPL25NLS-GFP-PRA Δ*kap123*-Import	BTY013	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal HTB2-CFP::HIS3MX TPI1-CFP::HIS3MX kap123::ura3::TRP1* pBT009 *(LEU2)*	BTY006 transformed with pBT009
RPL25NLS-GFP-PRA WT-Import	BTY014	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX HTB2-CFP::HIS3MX* pBT009 *(LEU2)*	BTY004 transformed with pBT009
RPL25NLS-YFP KAP121-CFP Δ*kap123*	BTY015	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT011 (LEU2)* pBT029 *(leu2 URA3)*	BTY001 transformed with pBT011 and pBT029
RPL25NLS-YFP KAP123-CFP Δ*kap123*	BTY016	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT011 (LEU2)* pBT030 *(leu2 URA3)*	BTY001 transformed with pBT011 and pBT030
RPS1BNLS-GFP Δ*kap123*	BTY017	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1* pBT013 *(LEU2)*	BTY001 transformed with pBT013
RPS1BNLS-GFP WT	BTY018	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* pBT013 *(LEU2)*	DF5a transformed with pBT013
MAK16-NLS-GFP Δ*kap123*	BTY022	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1* pBT015 *(LEU2)*	BTY001 transformed with pBT015
MAK16-NLS-GFP WT	BTY023	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* pBT015 *(LEU2)*	DF5a transformed with pBT015
PHO4NLS-GFP-PRA WT-Import	BTY024	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX HTB2-CFP::HIS3MX* pBT018 *(LEU2)*	BTY004 transformed with pBT018
NAB2NLS-GFP-PRA WT-Import	BTY025	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal TPI1-CFP::HIS3MX HTB2-CFP::HIS3MX* pBT016 *(LEU2)*	BTY004 transformed with pBT016
RPL25NLS-GFP Vector Δ*kap123*	BTY027	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT024 (leu2 URA3)* pBT008 *(LEU2)*	BTY001 transformed with pBT024 and pBT008
RPL25NLS-GFP KAP95-HA Δ*kap123*	BTY028	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT025 (leu2 URA3)* pBT008 *(LEU2)*	BTY001 transformed with pBT025 and pBT008
RPL25NLS-GFP KAP104-HA Δ*kap123*	BTY029	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT026 (leu2 URA3)* pBT008 *(LEU2)*	BTY001 transformed with pBT025 and pBT008
RPL25NLS-GFP KAP121-HA Δ*kap123*	BTY030	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT027 (leu2 URA3)* pBT008 *(LEU2)*	BTY001 transformed with pBT027 and pBT008
RPL25NLS-GFP KAP123-HA Δ*kap123*	BTY031	*MATa trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal* Δ*kap123::ura3::TRP1 pBT028 (leu2 URA3)* pBT008 *(LEU2)*	BTY001 transformed with pBT028 and pBT008

Table S3. **List of all Kap123p-interacting proteins from overlay assays**

Gene name	Kap123p binding[a]	Kap121p binding[a]	Function[b]
RPL18A	++++	++	Ribosomal protein
RPL18B	++++	++	Ribosomal protein
RPL25[c]	++	++	Ribosomal protein
RPL3	++++	++++	Ribosomal protein
RPL4A	++	++	Ribosomal protein
RPL4B	++	++	Ribosomal protein
RPL6A	++	++	Ribosomal protein
RPL6B	++	++	Ribosomal protein
RPS17A	++	+	Ribosomal protein
RPS17B	++	+	Ribosomal protein
RPS1A	+++	+	Ribosomal protein
RPS1B[c]	+++	+	Ribosomal protein
RPS4A	+++	+++	Ribosomal protein
RPS4B	+++	+++	Ribosomal protein
RPS8A	++++	+++	Ribosomal protein
RPS8B	++++	+++	Ribosomal protein
CBF5	+++	+++	Ribosome assembly
DBP7	+	+	Ribosome assembly
MAK16[c]	+++	++	Ribosome assembly
MAK5	+	+	Ribosome assembly
NHP2	+	+	Ribosome assembly
NOC2	++++	+++	Ribosome assembly
NUG1[c]	+++	+	Ribosome assembly
RLP7	++	++	Ribosome assembly
TSR1	++	+	Ribosome assembly
YIL096C[c]	+++	+	Ribosome assembly
RAI1	+++	+	RNA processing
UTP21	++	+	RNA processing
CTR9	+	+	Transcription
RPG1	+	+	Translation initiation
NCL1	++	+	tRNA modification
YHR127W	+++	+	Unknown
MSH2	+++	+++	DNA repair
SSB2	+++	+	Heat shock protein
MKT1	++++	++	Mating
ADE12	+++	+	Metabolic enzyme
YRA1[c]	++++	+	mRNA export
NIC96	++++	++	NPC protein
NUP60	+++	+	NPC protein

[a]Relative strength of Kap-binding signal estimated from overlay assays.
[b] As listed at the Yeast Proteome Database (http://www.biobase-international.com/).
[c] Chosen for further study.

Kap overexpression constructs were made under the *CUP1* promotor, in a variant of the pYEX-BX 2μ yeast expression vector (CLONTECH Laboratories, Inc.). This vector had been modified by the introduction of a frameshift mutation early in the *LEU2* marker gene, leaving only a functional *URA3* auxotrophic marker. Thus, these vectors could be cotransformed into cells with the *LEU2*-marked NLS-GFP expression vectors. *KAP95*, *KAP104*, *KAP121*, and *KAP123* were cloned with two HA tags encoded in their 3′ PCR primers and inserted into modified pYEX-BX. *KAP121* and *KAP123* were made as eCFP-tagged (CLONTECH Laboratories, Inc.) versions by encoding a cloning site in their 3′ PCR primers for insertion of eCFP.

Lysine-to-alanine mutations were introduced at amino acids 21 and 22 of *RPL25NLS-GFP* using the QuikChange II XL Site Directed Mutagenesis kit (Stratagene).

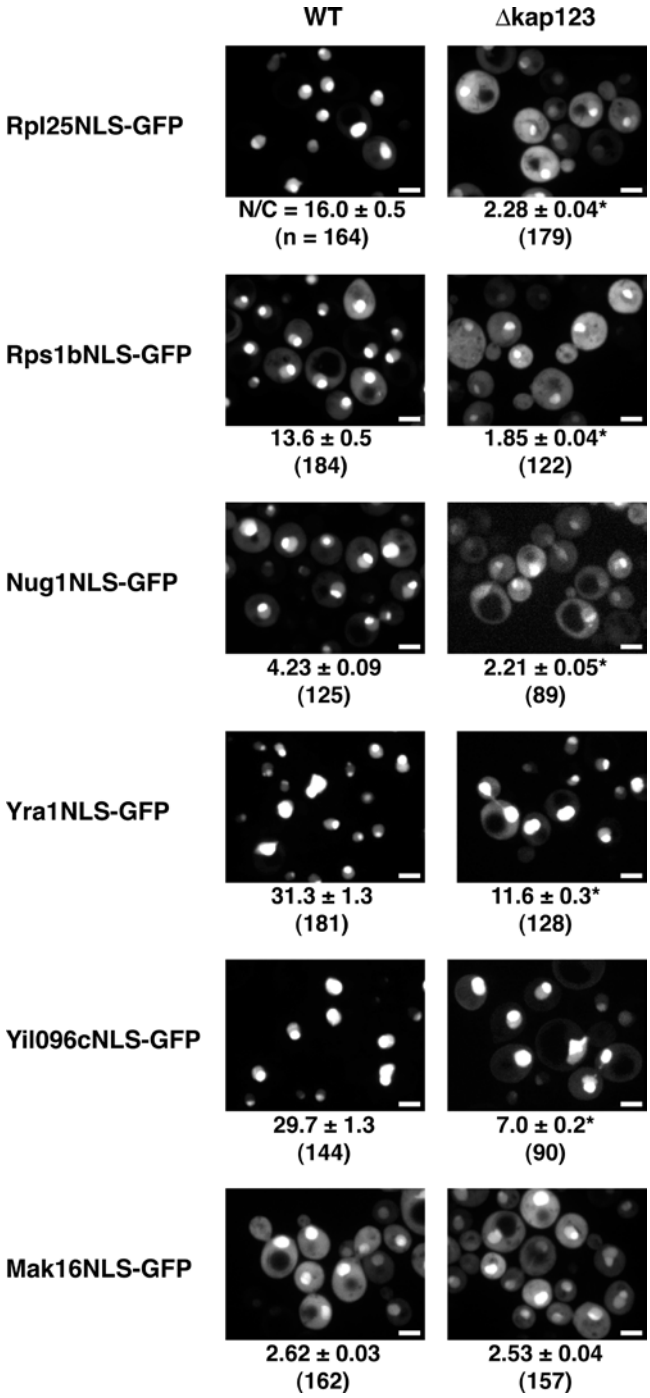

Figure S3. **Steady-state distributions of ribosomal NLS-GFP cargoes are significantly altered in *Δkap123* yeast.** NLSs of six putative Kap123p cargoes (Table 1) were expressed as NLS-GFP fusion proteins from 2μ plasmids in wild-type or *Δkap123* yeast. Fluorescence images of the resulting steady-state distributions of these cargoes are shown. Bars, 3 μm. The average nuclear-to-cytoplasmic ratio of each cargo in either strain was calculated from images of several cells, with uncertainty values of the SD of the mean. Cargoes that had a significantly different ratio in *Δkap123* cells than wild-type cells (*t* test P value of <0.0001) are marked with asterisks.

Plasmids containing *GST-KAP104* (Marelli et al., 1998) and *GST-KAP121* (Lee and Aitchison, 1999) were gifts from J. Aitchison (University of Washington, Seattle, WA), whereas *GST-KAP123-HA* was made for this study; all were constructed in pGEX vectors (GE Healthcare). Wild-type and mutant *RPL25NLS-GFP*, *NAB2NLS-GFP*, and *PHO4NLS-YFP* constructs were amplified by PCR from their respective yeast expression plasmids and inserted into pET21b (Novagen) for bacterial expression and purification as 6xHIS-tagged fusion proteins.

Strain construction

All strains and their derivations are described in Table S3.

The *URA3* marker of Δ*kap123* was changed to *TRP1* by transforming with the appropriate marker-swap plasmid (Cross, 1997). This allowed Δ*kap123* yeast to be transformed with *URA3*-marked Kap-expression vectors.

"Import" strains were constructed by standard C-terminal–tagging methodology (Aitchison et al., 1995). Genomic copies of *HTB2* and *TPI1* were tagged separately with a *CFP::HIS3MX* cassette that was a gift from A. Neiman (State University of New York, Stony Brook, NY; Nickas and Neiman, 2002). The WT-Import strain was made by mating *HTB2-CFP* and *TPI1-CFP* tagged strains, then selecting for haploid spores with the expected subcellular fluorescence distribution. The Δ*kap123*-Import strain was made by similar mating and selection, crossing the WT-Import strain with Δ*kap123::TRP1*.

All transformations were performed using the standard electroporation method (Grey and Brendel, 1992).

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