Supporting information to:

The mRNA export factor Npl3 mediates the export of large ribosomal subunits

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Running title: Npl3 Functions in Large Ribosomal Subunit Export

Supplementary information

Figure legends

Fig. S1| Npl3 is part of the exported mRNP. (A) Npl3 is essential in the *S288C* strain background. Serial dilutions of *S288C npl3*Δ either with an empty vector or with *NPL3* were spotted onto –URA plates or onto FOA-plates that select for the loss of the covering plasmid and incubated at 25°C for three days. (B) Npl3 is not essential in the *BY* strain background. Serial dilutions of *BY npl3*Δ were spotted onto YPD plates and incubated at 25°C or 37°C for three days. (C) Localization of an at steady state cytoplasmic mutant of *NPL3* (GFP-npl3$_c$), expressed in *BY*-wildtype or *-rat7-1 npl3*Δ cells that were grown to the logarithmic growth before shifted to 37°C for the indicated times. The DNA was stained with DAPI. (D) Ribosomal profiles showing the incorporation of Rpl25-GFP into ribosomes. Wildtype expressing Rpl25-GFP was grown to log phase before their lysates were applied onto 5-50% sucrose density gradients, fractionated and analyzed in flow through photometry. The fractions are shown in western blot analyses with antibodies against GFP (top). The same experiment was performed with the addition of RNase, which destroys the polysomes (bottom).

Fig. S2| *npl3*Δ has no obvious nucleolar export defects and rRNA processing defects. (A) Rpl25-GFP does not accumulate in the nucleolus. Co-localization experiments of the nuclear localization of Rpl25-GFP in *npl3*Δ with the DAPI staining and an immunofluorescence with antibodies against the nucleolar protein Nop1 are shown in log phase cells shifted to 37°C for 30 min. (B/C) *npl3*Δ cells have no obvious rRNA processing defects. Total RNA was extracted from wildtype and *npl3*Δ and examined by the Bio-Rad Experion^{TM}. (B) rRNA bands in a virtual gel image and electropherograms (C) are shown. The RNA quality was assessed by RQI (RNA quality indicator) values as well as the ratios between the 25S and 18S rRNA peaks (=rRNA ratio).

Fig. S3| The export of the small ribosomal subunit is independent of Npl3. (A) Localization of the small ribosomal subunit reporter protein Rps2-GFP is shown in wildtype, *npl3*Δ and *xpo1-1* in the *BY*-strain background at 25°C and upon temperature shift to 37°C for the indicated times. (B) A novel assay tracking single 18S rRNA molecules reveal no export defects for the small ribosomal subunit in *npl3*Δ. Localization of the DIG-UTP labeled 18S rRNA is shown in *in situ* hybridization experiments in wildtype, *npl3*Δ, *nmd3-2, mtr2-33, xpo1-1* and *rat7-1* in the *BY*-strain background at 25°C and upon temperature shift to 37°C for the indicated times.

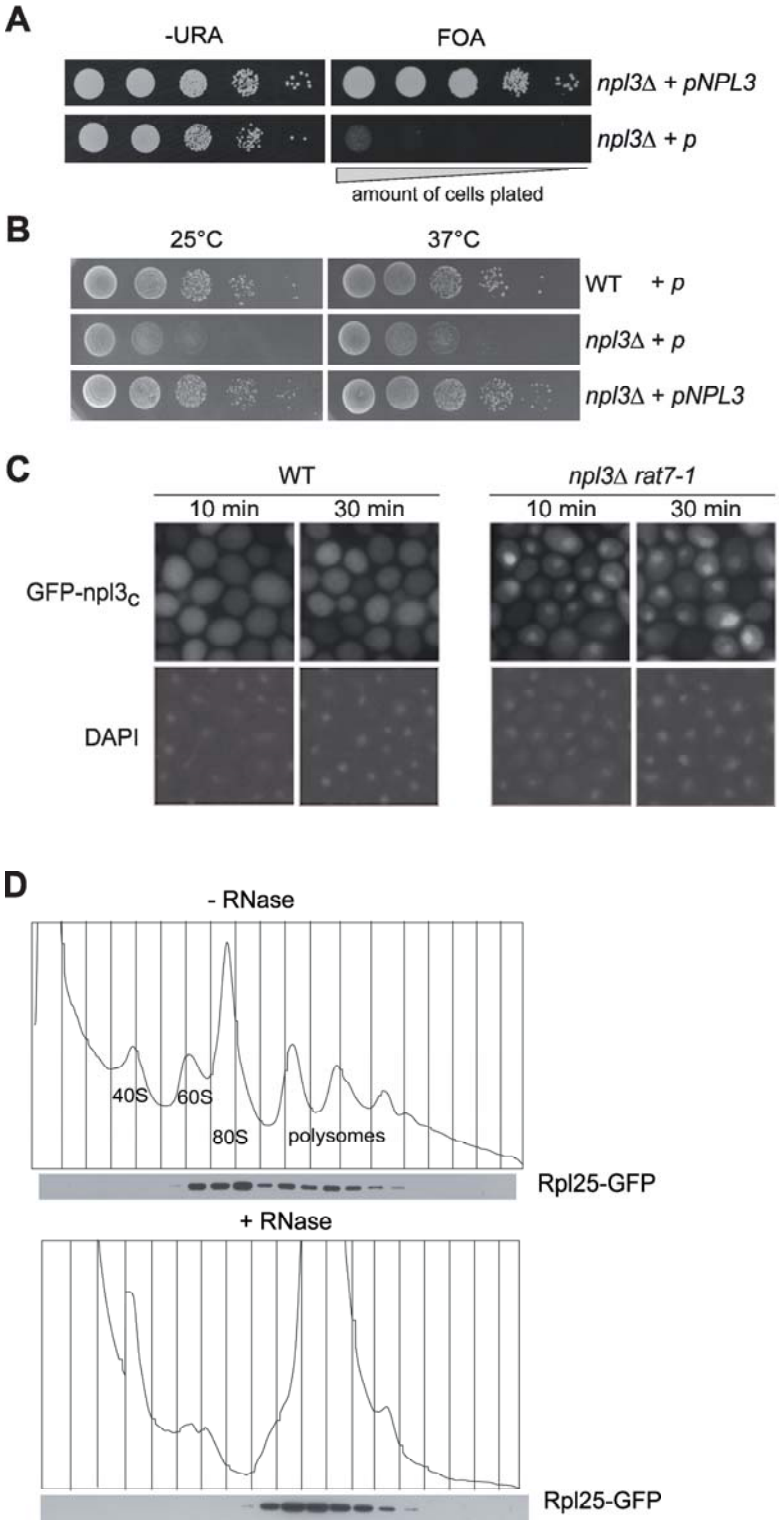
Fig. S4| Interaction of Npl3 with the large ribosomal subunit is not RNase sensitive and not restricted to the *BY*-strain background. (A) The interaction between Npl3 and Rpl25 shown in Fig 3A is not RNase sensitive in contrast to the interaction between Dbp5 and Pab1 shown here. Co-immunoprecipitations with Pab1-GFP were performed with Dbp5-myc. (B) Ethidiumbromide stained rRNA is shown on a 1.5% argarose gel, prepared from the lysates treated with or without RNase. (C) Co-immunoprecipitations of Npl3 were performed with GFP-tagged Rpl25 in *S288C*. Total lysates were split and incubated with or without RNase prior to elution. Western blot analyses with antibodies against GFP, Npl3 and Hem15 are shown.

Fig. S5| Npl3 interacts with FG-repeats. 10% Coomassie stained SDS-gel showing GST-pull downs of a GST-tagged GLFG-repeat domain of Nup116 with recombinantly expressed and purified Npl3 in the presence of competitive bacterial lysate.

Fig. S6| Model describing the transport functions of Npl3. mRNA export: Npl3 binds to the mRNA in the nucleus and recruits the heterodimer Mex67-Mtr2 for mRNA export. In this way Npl3 acts as an adapter for the export receptor heterodimer. Mex67-Mtr2 contacts the nuclear pore complex (NPC) during transit. Pre-60S ribosomal subunit export: For the export of the pre-60S ribosomal subunit Npl3 directly interacts with the 25S rRNA and Rpl25 on the

one side and with the NPC on the other side. In this way, Npl3 functions without Mex67 and acts rather as a receptor as it is able to directly interact with the NPC. Other pre-60S export receptors and their adapters such as Xpo1-Nmd3 and Mex67-Mtr2 interact with the pre-60S particle on their own. All three transporters together with auxiliary transport factors (not shown) act in concert to mediate the export of this large ribonucleoparticle (RNP).

Figure S1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-URA
FOA
npl3Δ + pNPL3
npl3Δ + p
amount of cells plated
B
25°C
37°C
WT + p
npl3Δ + p
npl3Δ + pNPL3
C
WT
npl3Δ rat7-1
10 min
30 min
10 min
30 min
GFP-npl3C
DAPI
D
- RNase
40S
60S
80S
polysomes
Rpl25-GFP
+ RNase
Rpl25-GFP

Figure S2

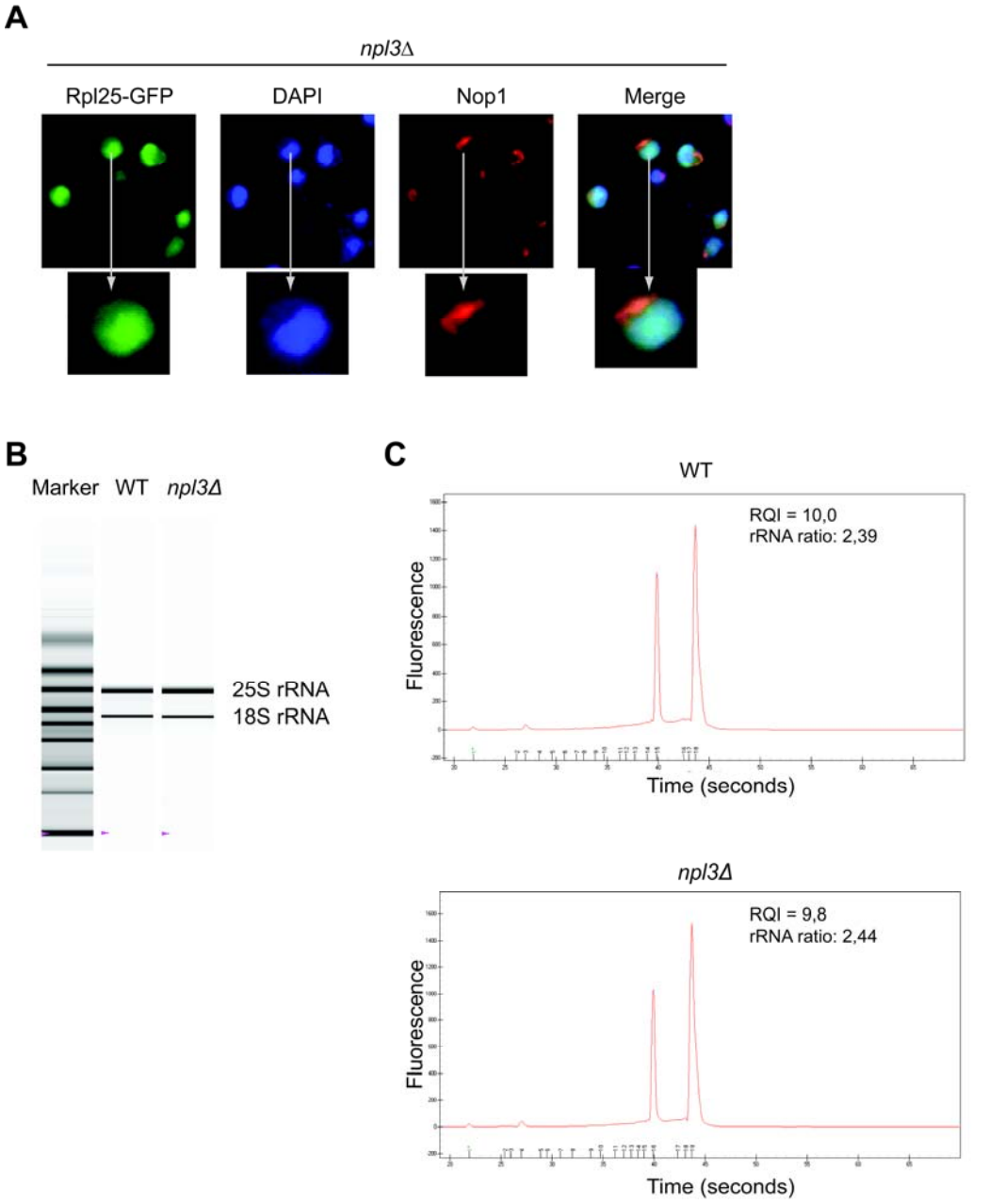

A
npl3Δ
Rpl25-GFP
DAPI
Nop1
Merge
B
Marker
WT
npl3Δ
25S rRNA
18S rRNA
C
WT
RQI = 10,0
rRNA ratio: 2,39
Fluorescence
Time (seconds)
npl3Δ
RQI = 9,8
rRNA ratio: 2,44
Fluorescence
Time (seconds)

Figure S3

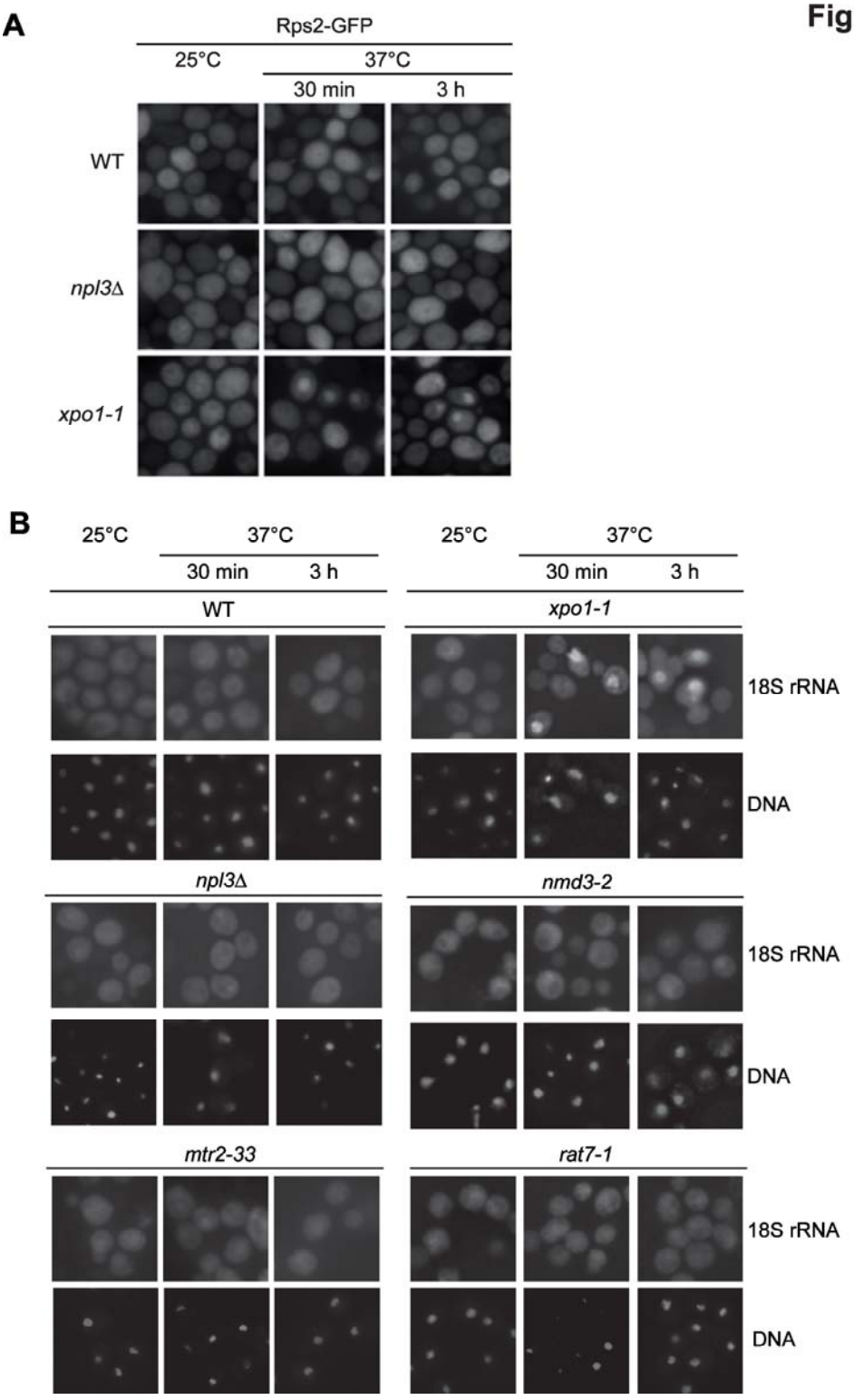
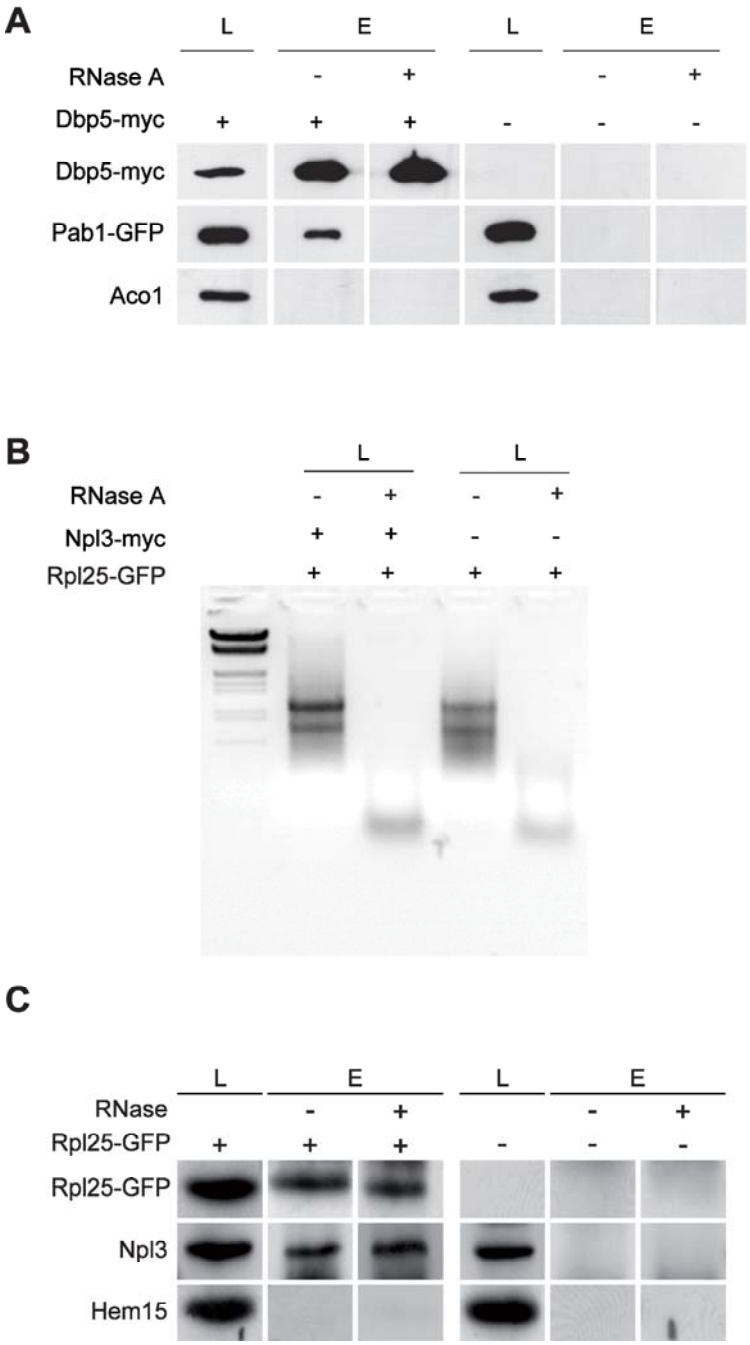

A
Rps2-GFP
25°C
37°C
30 min
3 h
WT
npl3Δ
xpo1-1
B
25°C
37°C
30 min
3 h
WT
xpo1-1
18S rRNA
DNA
npl3Δ
nmd3-2
18S rRNA
DNA
mtr2-33
rat7-1
18S rRNA
DNA

Figure S4


A
L
E
L
E
RNase A
-
+
-
+
Dbp5-myc
+
+
+
-
-
-
Dbp5-myc
Pab1-GFP
Aco1
B
L
L
RNase A
-
+
-
+
Npl3-myc
+
+
-
-
Rpl25-GFP
+
+
+
+
C
L
E
L
E
RNase
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Rpl25-GFP
Npl3
Hem15

Figure S5

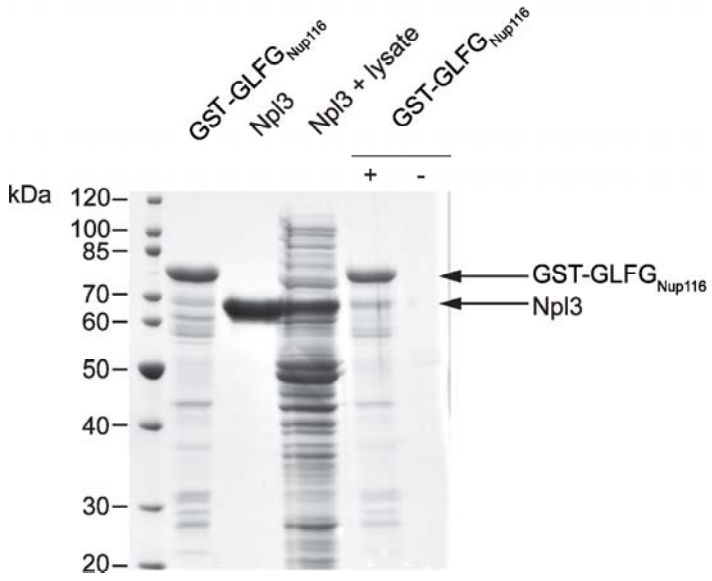
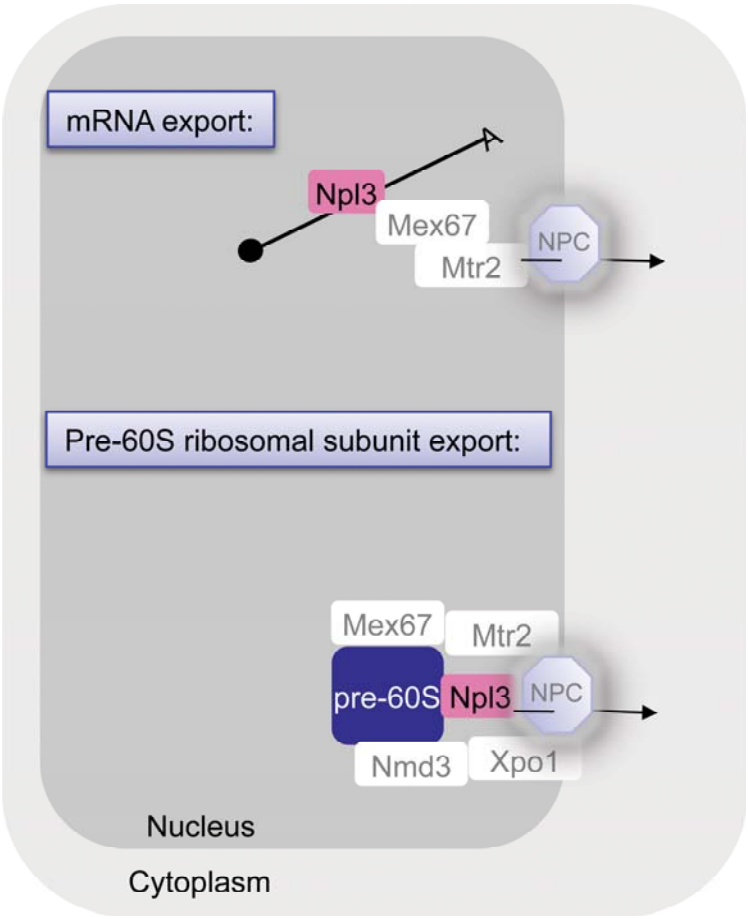


Figure S6



ADDITIONAL METHODS

Yeast Strains, Plasmids and Oligonucleotides. All yeast strains used in this study are listed in the Supplemental Table 1, plasmids are listed in Table 2 and oligonucleotides in Table 3. All experiments unless indicated differently were performed in the *BY-series* of the *Saccharomyces cerevisiae* strain background (Brachmann et al., 1998) that was used in the deletion project (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html) and that is listed in the *Saccharomyces* genome database, SGD (http://www.yeastgenome.org/). Strains were cultivated in standard media at 25°C. All growth tests are shown in 10-fold serial dilution starting with approximately 10^5 cells per drop.

Construction of plasmids and yeast strains

Construction of genomic RPL25-myc

Construction of the genomic *myc*-tagged *RPL25* was done according to Knop et al. (1999). The myc-insertion cassette was amplified by PCR with the primer set HK495 and HK496 from pHK492 and yeast cells were transformed with the PCR product. Correct integration was verified by PCR amplification of genomic DNA and Rpl25-myc protein expression.

Construction of plasmid encoded GFP-NPL3 (pHK765)

P_{Npl3}*GFP-NPL3* was excised from pHK418 with *Not*I/*Sal*I and inserted into *Not*I/*Sal*I restricted pHK88.

Construction of plasmid encoded 9 x myc-NPL3 (pHK778)

StuI restricted PCR of pHK493 with primer set HK553 and HK554 was ligated into StuI digested pHK418

Construction of the recombinant NPL3 expression plasmid

Coding sequences for the ORF of *NPL3* from pHK26 was PCR-amplified (HK181, HK652) and cloned via the TOPO TA cloning kit (Invitrogen) and *Bam*HI digestion into pET-15b resulting in a His-tagged *NPL3* (pHK845).

GFP Fluorescence

All light microscopy studies were performed with a Leica AF6000 microscope and pictures were obtained by using the Hamamatsu 1394 ORCA-ERA camera and the LAS AF 1.6.2 software (Leica). Export of ribosomal subunits by using the *RPL25-GFP* and *RPS2-GFP* reporter constructs were analyzed according to (Gadal et al., 2001; Milkereit et al., 2001). Localization studies of npl3-RA8-GFP (GFP-npl3$_c$) were performed according to (Gilbert et al., 2001a). For immunofluorescence studies, yeast cells were grown at 25°C in the appropriate medium to the log phase (1-3 x 10^7 cells/ml). If indicated a temperature shift to 37°C was done, before the cells were treated as described in (Krebber et al., 1999b). The Nop1-antibody was used in a 1:250 dilution.

Co-immunoprecipitation experiments

Cells were grown at 25°C to a density of OD_{600} 0.5-0.8 in the appropriate SD medium. If indicated a temperature shift to 37°C was done before 100-200 OD_{600} of the cells were collected, washed once in ddH_2O and resuspended in 600 µl of PBSMKT-buffer (137 mM NaCl, 5.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.5 mM $MgCl_2$, 0.05-0.5% (v/v) Triton X-100). Yeast cells were transferred to microcentrifuge tubes, one cell volume of glass beads and protease inhibitor cocktail (Sigma-Aldrich (Nr. P8215) and Roche Applied Science (Nr. 11873580001)) were added, and the cells were lysed by vigorous vortexing for 20 sec and 4.5 m/s using FastPrep®-24 (MP Biomedicals). After spinning for 5 min at 16.000 x g, the clarified supernatants were either directly used or split into two equal portions (+/- addition of RNase A) if indicated and incubated for 2-4 h at 4°C with gentle agitation either with 10 µl of Protein G sepharose beads (Amersham Biosciences) or with 20 µl Protein G agarose beads (Santa Cruz), conjugated to monoclonal c-myc (9E10)- or polyclonal GFP-specific antibodies. In the RNase containing samples the enzyme was added to the PBSMKT-buffer to a final concentration of 200 µg/ml. The beads were washed five times with 1 ml PBSMKT-buffer by repeated low speed centrifugation (600 x g). Samples of the lysates, the final washes and the eluates were mixed with 2 x SDS sample loading buffer and separated on 10-15% (w/v) SDS-PAGEs.

Purification of recombinant proteins

Recombinant proteins were expressed in *E. coli* (BL21 DE3). Cells were induced with 1 mM IPTG for 4 h at 37°C. All following steps were done on ice or 4°C.

Cells expressing His-Npl3 were lysed in binding buffer (150 mM NaCl, 30 mM HEPES, 5% glycerol, 2 mM β-mercaptoethanol, 20 mM imidazole; adjusted to pH8.0) and after spinning for 30 min at 20000 x g, the lysate was filtered by a syringe filter and loaded onto a HisTrap FF column (Ni Sepharose 6 Fast Flow from GE Healthcare). The whole purification was done with an ÄKTAprime^{TM}plus (GE Healthcare). After binding to the column it was washed with binding buffer and finally the protein was eluted with elution buffer (150 mM NaCl, 30 mM HEPES, 5% glycerol, 2 mM β-mercaptoethanol, 500 mM imidazole; adjusted to pH8.0). Protein size and potential contaminations were controlled by SDS gel electrophoresis. The eluted His-Npl3 was dialysed against a storage buffer (150 mM NaCl, 20 mM HEPES pH7.5, 5% glycerol, 2 mM DTT) and concentrated via Vivaspin ultrafiltration spin columns (Sartorius). Aliquots were stored at -80°C.

Cells expressing GST-GLFG$_{Nup116}$ were lysed in PBS buffer (pH7.3) plus 2 mM DTT and 1 mM PMSF, using a fluidizer (Microfluidics). After centrifugation and syringe filtration the lysate was loaded onto a GSTrap (FF) column (GE Healthcare). Purification was performed on an ÄKTAprime^{TM} (GE Healthcare) at flow rate of 1ml/min. After loading, the column was washed with four column volume of PBS pH7.3 plus 2 mM DTT and 10 mM PMSF. The GST-protein was eluted with elution buffer (PBS pH7.3, 2 mM DTT, 30 mM reduced glutathione). The eluted GST-GLFG$_{Nup116}$ fractions were pooled and dialyzed against storage buffer. Aliquots were stored at -80°C.

***In vitro* RNA co-immunoprecipitation experiments**

Recombinant His-Npl3 was equilibrated with RNA-binding buffer (25 mM Tris-HCl pH 7.4, 100 mM KCl, 0,2% (v/v) Triton X-100, 0,2 mM PMSF, 5 mM DTT, 10 units RiboLock™ RNase Inhibitor (Fermentas)) and incubated with fragments of the 25S, 5.8S or 18S rRNAs. *In vitro* transcription of rRNA

fragments was performed using purified PCR products, which were generated by PCR amplification of rDNA with primers harboring a T7 transcription site. 10 µg of recombinant Npl3 (rNpl3) and 1 µg of *in vitro* transcribed rRNA was incubated with 15 µl of Protein G-sepharose beads (GE Healthcare) conjugated to a specific Npl3-antibody for 1 h at 4°C in RNA-binding buffer supplemented with protease inhibitor cocktails (Sigma-Aldrich (Nr. P8215) and Roche Applied Science (Nr. 11873580001)). Upon immunoprecipitation, beads were washed 5 times with RNA-binding buffer and RNA was eluted in 100 µl DEPC-treated ddH_2O during incubation at 65°C for 10 minutes. The RNA eluate was purified via phenol-chloroform extraction and ethanol precipitation and it was finally eluted in 10 µl of DEPC-treated ddH_2O. Binding of the 25S, 5.8S or 18S rRNA fragments was analyzed by using RT-PCR with the specific primer pairs HK986/HK674, HK985/HK688 and HK983/HK666.

***In vivo* RNA co-immunoprecipitation**

Yeast cells were grown at 25°C in selective medium to a density of ~ 3 x 10^7 cells/ml. The cells were collected, washed once with 1 x TBS and resuspended in 700 µl RNA-binding buffer. One cell volume of glass beads and protease inhibitor cocktails (Sigma-Aldrich (Nr. P8215 and Roche Applied Science (Nr. 11873580001) were added. Cells were lysed by vigorous vortexing for 30 sec and 4 m/s using the FastPrep-24 instrument (MP Biomedicals). For DNaseI digestion 700 µl lysate was incubated with 15 µl DNaseI (Qiagen, 40 Kunitz units) at 30°C for 30 min. The co-immunoprecipitation was performed at 4°C for 4 h by incubating lysates with 20 µl Protein G sepharose beads conjugated to monoclonal GFP-antibody (Santa Cruz). The beads were washed five times with RNA-binding buffer and associated RNA was eluted in 100 µl DEPC treated ddH_2O during incubation at 65°C for 10 min. The RNA eluate was purified via phenol-chloroform extraction and ethanol precipitation. RNA was finally eluted in 10 µl of DEPC treated ddH_2O and used for qRT-PCR.

Reverse transcription (RT) and quantitative PCR (qPCR)

1 µg of the RNA eluate from the *in vivo* RNA-co IP experiments was reverse

transcribed using nonamer primers and the Omniscript kit (Qiagen). With the Rotor-Gene™ SYBR Green PCR kit (Qiagen) and a primer concentration of 0,2 µM PCR reactions were conducted following the manufacturers instruction. qRT-PCR was performed in a Rotor-Gene® qPCR machine (Qiagen, Hilden, Germany) for 40 cycles at an annealing temperature of 60°C. All amplifications were performed in triplicates and three biological replicates. The triplicate mean values were calculated according to the ΔΔCt quantification method using the untagged wildtype control as a reference for normalization.

rRNA *in situ* hybridization

For synthesis of DIG-UTP labeled rRNA probes, 250 ng purified PCR templates (HK671/HK975 for 25S rRNA and HK667/HK976 for 18S rRNA) were generated including a T7 transcription site on an antisense strand. *In vitro* transcription of the antisense RNA was performed using T7 RNA Polymerase (Fermentas) and DIG-UTP RNA labeling Mix (Roche) according to the standard protocol. DIG labeled antisense transcripts were precipitated with 1/4th 4 M LiCl, 1 µl glycogen and 2.5 Vol. EtOH and resuspended in 0,5 x TE/formamide/hybmix (50% formamide, 5 x SSC, 5 x Denhardts, 250 µg/ml tRNA, 500 µg/ml salmon sperm DNA, 50 µg/ml heparin, 2,5 mM EDTA pH8.0, 0,1% Tween-20). For rRNA *in situ* hybridization cells were grown to 2 x 10^7 cell/ml at 25°C and shifted to 37°C for the indicated time. Cells were fixed by adding formaldehyde to a final concentration of 4% and incubated for 1 h at 37°C. Cells were collected and washed twice with 0,1 M phosphate buffer pH6.5 and once with P-solution. Cells were zymolase treated, permeabilized and then prehybridized with hybmix for 1 h on a polylysine coated slide as described in (Krebber et al. 1999). After hybridization over night at 37°C with the respective digoxigenin-labeled rRNA probe, cells were washed with 0,5 to 2 x SSC/50% deionized formamide. Cells were blocked in antibody blocking buffer for 1 h. A sheep anti-digoxigenin Fab-FITC antibody (Roche) was used at a 1:200 dilution and incubated over night at 37°C. After washing with Aby wash I buffer, nuclei were stained with DAPI as described in (Krebber et al. 1999). Microscopy studies were performed with a Leica AF6000 microscope

and pictures were obtained by using the Hamamatsu 1394 ORCA-ERA camera and the LAS AF 1.6.2 software (Leica).

Sucrose gradient experiments

Cells were grown in selective media to log-phase at 25°C. After a temperature shift for 90 min to 37°C the cells were incubated with 200 µg/ml cycloheximide for 20 min on ice. Every following step was done on ice or 4°C. Cells were lysed in lysis buffer (20 mM Tris pH7.4, 20 mM KCl, 5 mM $MgCl_2$, 200 µg/ml cycloheximide, 12 mM mercaptoethanol, Roche protease inhibitor without EDTA) by adding glass beads and vortexing for 20 sec using the FastPrep®-24 (MP Biomedicals). Crude extracts were centrifuged for 15 min at 16000 x g. The supernatant (each sample 800 µg total protein) was loaded onto a 6% - 48% linear sucrose gradient (20 mM Tris pH7.4, 20 mM KCl, 5 mM $MgCl_2$). After centrifuging for 4 h at 36000 rpm in Beckman SW40 rotor, the gradient was fractionated and absorbance at 254 nm was measured.

***In vitro* protein-protein binding**

Gluthatione Sepharose™ 4B beads (GE Healthcare) were equilibrated for 1 h in binding-buffer (20 mM HEPES-KOH pH6.8, 150 mM KOAc, 2 mM $Mg[OAc]_2$, 0,1% Tween 20, 5% glycerol, 2 mM DTT, protease inhibitor cocktail without EDTA from Roche Applied Science) and blocked with 1% (w/v) BSA. Afterwards beads were washed 4 times with binding-buffer. 10 µg GST-$GLFG_{Nup116}$ was immobilized on 30 µl Gluthatione Sepharose™ 4B beads (GE Healthcare) and incubated on a rotator for 1 h at 4°C in a total volume of 400 µl binding-buffer. One sample without bound GST-protein served as a negative control. 10 µg of recombinant purified Npl3 was mixed with 15 µg *E. coli* lysate and added to all samples followed by an incubation for 1,5 h under continuous end over end rotation at 4°C. The beads were washed 4 times with binding-buffer by low speed centrifugation (600 x g). Beads were mixed with 2 x SDS sample loading buffer and separated on 10% (w/v) SDS-PAGEs. Bound GST protein was detected with anti-GST antibody at 1:3000 dilution and co-purified Npl3 with anti-Npl3 antibody (1:5000).

SUPPLEMENTARY TABLES

Table S1. Yeast strains

Number	Name	Genotype	Source
HKY36	FY86	*MATα ura3-52 leu2Δ1 his3-200*	Fred Winston
HKY157	PSY1641	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN URA3 NPL3*	Lee et al., 1996
HKY158	PSY906	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN LEU2 npl3-1*	Lee et al., 1996
HKY159	PSY907	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN LEU2 npl3-3*	Lee et al., 1996
HKY160	PSY908	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN LEU2 npl3-17*	Lee et al., 1996
HKY161	PSY912	*MATa ura3-1 leu2 his3 lys2 ade2 ade3 npl3::HIS3 + p CEN LEU2 npl3-41*	Lee et al., 1996
HKY280	FSY978	*MATa ura3-52 leu2 trp1 pep4-3 pre1-407 prb1-1122*	Zenklusen et al., 2001
HKY380	Y04268	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 npl3::kanMX4*	Euroscarf
HKY381	BY4742	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Euroscarf
HKY611		*MATα ura3 leu2 his3 npl3::kanMX4 rat7-1*	this study
HKY705		*MATa ura3 leu2 his3 trp1 rix1-1*	Bassler et al., 2001
HKY725		*MATa ura3Δ0 leu2Δ0 his3Δ0 lys2Δ0 xpo1::kanMX4 + p CEN HIS3 xpo1-1*	this study
HKY802		*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 RPL25-3xmyc:HIS3MX6*	this study
HKY805		*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 npl3::kanMX4 RPL25-3xmyc:HIS3MX6*	this study
HKY822		*MATa ura3Δ0 leu2Δ0 his3Δ1 npl3::kanMX4 xpo1::kanMX4 + p CEN URA3 NPL3 + p CEN HIS3 xpo1-1*	this study
HKY890		*MATa ura3 leu2 his3 trp1 ade2 mtr2::HIS3 + p CEN TRP1 mtr2-21 + p CEN URA3 MTR2*	Bassler et al., 2001
HKY892		*MATa ura3 leu2 his3 trp1 ade2 mtr2::HIS3 + p CEN TRP1 mtr2-33 + p CEN URA3 MTR2*	Bassler et al., 2001
HKY894		*MATα ura3 leu2 his3 lys2 trp1 nmd3::kanMX4 + p CEN TRP1 nmd3-2 + p CEN URA3 NMD3*	Bassler et al., 2001
HKY896		*MATa ura3 leu2 his3 trp1 npl3::kanMX4 mtr2::HIS3 + p CEN TRP1 mtr2-21 + p CEN URA3 MTR2*	this study
HKY897		*MATa ura3 leu2 his3 trp1 npl3::kanMX4 mtr2::HIS3 + p CEN TRP1 mtr2-33 + p CEN URA3 MTR2*	this study
HKY904		*MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 trp1::kanMX4 npl3::kanMX4 nmd3::kanMX4 + p CEN TRP1 nmd3-2 + p CEN URA3 NMD3*	this study

HKY906	*MATα ura3Δ0 leu2Δ0 his3Δ0 trp1::kanMX4 mtr2::kanMX4 + p CEN TRP1 mtr2-21 + p CEN URA3 MTR2*	this study
HKY908	*MATα ura3Δ0 leu2Δ0 his3Δ1 trp1::kanMX4 mtr2::kanMX4 + p CEN TRP1 mtr2-33 + p CEN URA3 MTR2*	this study
HKY914	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 trp1::kanMX4 nmd3::kanMX4 + p CEN TRP1 nmd3-2 + p CEN URA3 NMD3*	this study
HKY951	*MATa ura3 leu2 his3 trp1 RIX1-GFP:HISMX6*	Nissan et al., 2004
HKY953	*MATa ura3 leu2 his3 trp1 ARX1-TAP:TRP1*	Nissan et al., 2004
HKY1004	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 PRP43-TAP:HISMX6*	Open Biosystems
HKY1061	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 NUP60-TAP:HISMX6*	Open Biosystems
HKY1083	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 RPS9A-GFP:HISMX6*	Invitrogen
HKY1092	*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 nup60::kanMX4*	Euroscarf
HKY1096	*MATa ura3Δ0 leu2Δ0 his3Δ1 npl3::kanMX4 PRP43-TAP:HISMX6*	this study
HKY1097	*MATa ura3Δ0 leu2Δ0 his3Δ1 npl3::kanMX4 nup60::kanMX4 + p CEN URA3 NPL3*	this study
HKY1098	*MATα his3Δ leu2Δ ura3Δ rat7-1*	this study
HKY1099	*MATα his3Δ1 leu2Δ0 ura3Δ0 nmd3::kanMX4 p CEN LEU2 NMD3-GFP*	this study

Table S2. Plasmids

Number	Name	Features	Source
pHK20		*CEN LEU2 MEX67-GFP*	Santos-Rosa et al., 1998
pHK26		*CEN URA3 NPL3*	this study
pHK87	pRS315	*CEN LEU2*	Sikorski & Hieter, 1989
pHK88	pRS316	*CEN URA3*	Sikorski & Hieter, 1989
pHK103	pRS425	*2µ LEU2*	Sikorski & Hieter, 1989
pHK104	pRS426	*2µ URA3*	Sikorski & Hieter, 1989
pHK195	pPS1152	*2µ URA3 NPL3*	Lee et al., 1996
pHK260		*2µ LEU2 RAT8-myc*	Snay-Hodge et al., 1998
pHK285		*2µ LEU2 MTR2*	Santos-Rosa et al., 1998
pHK418	pCS38	*CEN LEU2 GFP-NPL3*	Gilbert et al., 2001
pHK419	pCS55	*CEN LEU2 GFP-npl3c (npl3-RA8)*	Gilbert et al., 2001
pHK492	pYM5	*3xmyc-HIS3MX6*	Knop et al., 1999
pHK493	pYM6	*9xmyc-klTRP1*	Knop et al., 1999
pHK526	pSW304	*GST-GLFG*$_{Nup116}$	Iovine & Wente, 1997
pHK697		*CEN URA3 RPS2-GFP*	Milkereit et al., 2003
pHK698		*CEN URA3 RPL25-GFP*	Gadal et al., 2001
pHK705		*CEN TRP1 mtr2-21*	Bassler et al., 2001
pHK706		*CEN TRP1 mtr2-33*	Bassler et al., 2001
pHK708		*CEN TRP1 nmd3-2*	Bassler et al., 2001
pHK719	pAJ410	*2µ LEU2 NMD3*	Hedges et al., 2005
pHK720	pAJ538	*CEN LEU2 NMD3-myc*	Hedges et al., 2005
pHK721	pAJ582	*CEN LEU2 NMD3-GFP*	Hedges et al., 2005
pHK765		*CEN URA3 NPL3-GFP*	this study
pHK778		*CEN LEU2 9xmyc-NPL3*	this study
pHK845	pET-15b	6 x *HIS-NPL3*	this study

Table S3. Oligonucleotides

Number	Sequence
HK181	5’-GGATCCTTACCTGGTTGGTGATCTTTC-3’
HK495	5’-GCTGACTACGATGCTTTGGACATTGCTAACAGAATCGGTTACATTCGTACGCTGCAGGTCGAC-3’
HK496	5’-AAAAATTTAAAAATAATATTAAATTTATTAATTAAACCAATTAGAATCGATGAATTCGAGCTCG-3’
HK553	5’-AAAAGGCCTATGGGTGAACAAAAGTTGATTTC-3’
HK554	5’-TTTAGGCCTTCCGTTCAAGTCTTCTTC-3’
HK652	5'-CGCGGATCCCATGTCTGAAGCTCAAGAAAC-3'
HK653	5'-CCTTAGTAACGGCGAGTGAAGCGG-3'
HK654	5'-GGAATTTACCACCCACTTAGAGCTGC-3'
HK655	5'-GGTTGATCCTGCCAGTAGTCATATGC-3'
HK656	5'-CCAGCACAAGGCCATGCGATTC-3'
HK663	5'-CGCATCGATGAAGAACGCAGCG-3'
HK664	5'-GCTTAAGTTCAGCGGGTACTCCTACC-3'
HK666	5'-CTTTAATGATCCTTCCGCAGGTTC-3'
HK667	5'-GAAGACTAACTACTGCGAAAGC-3'
HK671	5'-GTGGGCCATTTTTGGTAAGC-3'
HK674	5’-GGCTACCTTAAGAGAGTCATAGTTAC-3’
HK687	5'-AAACTTTCAACAACGGATCTC-3'
HK688	5'-AAATGACGCTCAAACAGGC-3'
HK983	5'-TAATACGACTCACTATAGGGGATAGTTATCTGGTTGATCCTGCC-3'
HK985	5'-TAATACGACTCACTATAGGGAAACTTTCAACAACGGATCTC-3'
HK986	5'-TAATACGACTCACTATAGGGCCTTAGTAACGGCGAGTGAAGCGG-3'
HK975	5'-TAATACGACTCACTATAGGGGGCTACCTTAAGAGAGTCATAGTTAC-3'
HK976	5'-TAATACGACTCACTATAGGGCTTTAATGATCCTTCCGCAGGTTC-3'
HK979	5'-CCCTGCCCTTTGTACACACC-3’

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