SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Media and growth conditions. All strains were grown at 30°C (unless otherwise indicated) in rich, 5-fluoroorotic acid (5FOA) or drop-out media containing 2% glucose, 1% raffinose, or 1% galactose (Kaiser et al., 1994).

Strains. The *NMD3-GFP* genomic fusion was made by homologous recombination as described (Longtine et al., 1998). The oligonucleotide primers 5'AGAAGATGGAGTC GAGAACACACCCGTTGAATCTCAGCAGCGGATCCCCGGGTTAATTAA and 3'-ACTGAAATGCTAGTGTTTGTTAAGAGTATATACTACTCTCGAATTCGAGCTCG TTTAAAC were used in PCR to amplify plasmid pFA6a-KanMX6-GFP(S65T). The PCR product was transformed into the wild-type haploid CH1305. Geneticin-resistant transformants were selected, and integration was confirmed by PCR and fluorescence microscopy to yield the strain AJY1708. AJY1548, a haploid strain containing a point mutation in genomic *CRM1* that renders it sensitive to the drug LeptomycinB (LMB), was produced as follows. A *crm1::KanMX/CRM1* heterozygous diploid (Research Genetics) was transformed with a derivative of pDC-CRM1(T539C) (Neville and Rosbash, 1999) in which *LEU2* was replaced with *URA3*. Cells were sporulated, and a *crm1::KanMX* containing spore clone was transformed with the *Bgl*II-*Sca*I *CRM1(T539C)*-containing fragment from pDC-CRM1(T539C) (Neville and Rosbash, 1999). Transformants were incubated on YPD plates overnight and then replicated twice to 5FOA-containing plates. 5FOA-resistant clones were scored for G418 sensitivity and integration was confirmed by PCR analysis utilizing the HA-tag within *CRM1(T539C)*.

AJY1708 was then crossed with AJY1548, and the resulting heterozygous diploid was sporulated to give the spore clone AJY1705 (*NMD3-GFP crm1[T539C]-HA*). AJY1896 (*nmd3::TRP1 crm1[T539C]-HA*) was generated by mating AJY1548 against AJY531 (*nmd3::TRP1*). The resultant diploids were sporulated and dissected to yield a spore clone possessing both of the altered loci (*nmd3::TRP1 crm1[T539C]*). *nmd3-3 lsg1-2* and *nmd3-4 lsg1-3* strains were generated in the following manner. AJY729 and AJY736, haploid strains containing genomically-integrated *nmd3-3* and *nmd3-4* mutant alleles (alternate spore clones of those described previously) (Ho and Johnson, 1999), respectively, were mated against AJY1171 (*lsg1::KanMX4*), possessing a wild-type copy of *LSG1* on a pRS316 vector (pAJ626) (Kallstrom et al., 2003). The resulting heterozygous diploids were sporulated to yield spore clones AJY1511 (*lsg1::KanMX4* [pAJ626]), AJY1512 (*lsg1::KanMX4 nmd3-3::HIS3* [pAJ626]), AJY1513 (*nmd3-3::HIS3*), and AJY1518 (*nmd3-4::HIS3*) and AJY1521 (*lsg1::KanMX4 nmd3-4::HIS3* [pAJ626]). AJY1511, AJY1512, and AJY1521 were transformed with either pAJ741 (*lsg1-2*) or pAJ742 (*lsg1-3*) (Kallstrom et al., 2003), and pAJ626 was replaced by the appropriate mutant *lsg1* allele by streaking transformants onto 5-FOA plates. An *rpl10::KanMX4* haploid (Research Genetics) containing pDEGQ2 was mated with AJY1705 to make AJY1836 (*MAT*a *NMD3-GFP::KanMX6 crm1[T539C] rpl10::KanMX4* (pDEGQ2)). The following scheme was used to make AJY1657 (*MAT*a *rpl10[G161D]*). *Rpl10[G161D]* in the integrating vector pRS406 was made by three-part ligation. The 5'-portion of *RPL10* was amplified by PCR using AJO264, 5'-CGCGGATCCGAAACTAGTTAGCAC, and the mutagenic primer 5'-GCCTGATCAG

GGAACTTGTATCTGG. The 3'-portion of *RPL10* was amplified using the mutagenic primer 5'-CTGCCTGATCAACAAAAGATTATTTTGTC and AJO268, 5'-CGCGGATCCTACCCAACATGCTGAAC. The mutagenic primers introduced the G161D mutation as well as a silent BclI site. The PCR products were digested with SpeI and BclI (5'-product) and BclI and HindIII (3'-product) and ligated into pAJ735 digested with SpeI and HindIII to give pAJ736. pAJ735 was made by amplifying *RPL10* from CH1305 genomic wild-type DNA using AJO264 and AJO268, digesting with BamHI, filling in the ends and ligating into PvuII-cut pRS406. *RPL10* alleles were confirmed by sequencing. pAJ736 was digested with BsaB1 and transformed into wild-type CH1305. Ura^+ transformants were screened by PCR and BclI-digestion for integration of *rpl10[G161D]*. Correct integrants were mated to the wild-type strain AJY1168 (Research Genetics). Pop-outs were selected on 5FOA-containing media and screened for retention of the *rpl10[G161D]* allele by PCR and BclI digestion. Heterozygous diploids were then sporulated and tetrads dissected to give AJY1657.

Construction of plasmids.

Unless otherwise noted, myc denotes 13 tandem copies of the c-myc epitope. pAJ1143 **(***GAL1::NMD3)* was obtained as a high-copy suppressor of *LSG1(N173Y,L176S)* (this work) in a screen using a *GAL1* yeast cDNA library (Liu et al., 1992). pAJ582 (*NMD3-GFP*) was made by amplifying *GFP* with 5' oligo AJO230 (AGAAGATGGAGTCGAGAACACACCCGTTGAATCTCAGCAGCGGATCCC GGG TTAATTAA) and 3' oligo AJO307 (GCGAAGCTTGGCCTCGAAACGTGAGTC) using pFA6a-GFP(S65T)-KanMX6 (Kaiser et al., 1994) as template, digesting with PacI and HindIII and ligating it into the same sites of pAJ538. Randomly mutagenized *NMD3*

suppressor mutants of *rpl10(G161D)* were made by pooling 20 separate 20 cycle PCR reactions using Taq polymerase (GeneChoice) with 5' oligo AJO106 (GCCGCTCGAGACACCATGGAATTCACACCTATAG), 3'oligo AJO305 (TCCCCCGGGCTGCTGAGATTCAACGGG) and CH1305 genomic DNA as template. The product was cotransformed into AJY1657 with Bsg1-gapped pAJ538 for *in vivo* homologous recombination. Plasmid-borne suppressors, including *NMD3[I112T, I362T]* (pAJ1315), were identified as fast-growing colonies at 35°C. The *NMD3[V340D]* mutant (pAJ1299) was similarly isolated from a PCR mutagenized library using by screening for 5FOA-sensitive clones in an *nmd3::HIS3* strain. pAJ410 (*NMD3*) was made by moving the SmaI-HindIII fragment from pAJ123 and ligated into pRS425.

To make pAJ879 (*GAL10*::*myc-LSG1*), containing a single myc tag, the NcoI-NheI fragment from pAJ289 was ligated into the same sites of pAJ368. pAJ409 was constructed by moving *NMD3* as a SmaI-HindIII fragment from pAJ123 into the same sites of pRS416. pAJ907 and pAJ908 were made by insertion of the *RPL25-eGFP* ORF from pASZ11-RPL25-eGFP into pRS415 and pRS416 respectively. pAJ363 (*NMD3* on *2μ* plasmid) was constructed by transferring the open reading frame of *NMD3* as a *SnaB*I-*Sal*I digested fragment from pAJ78 into the same sites of a derivative of pRS426 that also contained *ADE3*. To make pAJ415 (*NMD3[L291F]*), inverse PCR was carried out with the 5' oligo AJO303 (GCGGATCTGTCACCATCT) and the mutagenic 3' oligo AJO304 (GGTTTGGAAAGTAGTCGGGTCCATAAACTG) to incorporate the mutations underlined into the *NMD3* ORF (the second mutation, which is silent, was used to eliminate an internal BamHI site). The BglII to MscI fragment containing the L291F mutation was then used to replace the corresponding fragment of pAJ123 and pAJ1002 to

give pAJ415 and pAJ1070, respectively. pAJ1069 (*NMD3[L291F]AAA-GFP*) was made by three-part ligation of BglII-PacI and PacI-HindIII fragments from pAJ755 and BglII-HindIII cut pAJ415. To make pAJ754 (*NMD3AAA-GFP*), 5' oligo AJO247 (CGGAATTCACTGTCCAGTTTATGGATC) and 3' oligo AJO389 (5'GCAAGCTTAGATTAATGTCATTTCATCTGCCTCGTCGGCTAATTCATCAGC GTTGATTT) were first used in PCR to amplify nt843-1516 of *NMD3* while introducing missense mutations as underlined. This product was amplified with AJO247 and 5' oligo AJO413(GGCAAGCTTAGTTAATTAACCCGGGGATCCGCTGCTGAGATTCAACG GGTGTGTTCTCGACTCCATCTTCTAATGTCATTTCAT). The product was cut with BglII-PacI and ligated into BglII-PacI cut pAJ535 to make pAJ752. GFP, amplified from pFA6a-GFP(S65T)-KanMX using 5'-oligo AJO230 (AGAAGATGGAGTCGAGA ACACACCCGTTGAATCTCAGCAGCGGATCCCCGGGTTAATTAA) and AJO307 (GCGAAGCTTGGCCTCGAAACGTGAGTC) was cut with PacI-HindIII and ligated into the same sites of pAJ752. pAJ690 was made by inserting *NMD3* on a XhoI(filled in)-HindIII fragment from pAJ118 into the SmaI-HindIII sites of pEG(KT) (Mitchell et al., 1993). pAJ698, derived from pAJ690 contains an nmd3 allele deleted of 194 C-terminal amino acids. PAJ1291, pAJ1292 and pAJ1293 were made by subcloning fragments from pAJ1070, pAJ1315 and pAJ1299, respectively, into pAJ698. pAJ1287 (*NMD3[I112T,I362T]-GFP*) was constructed by ligating the BglII/PacI and PacI/HindIII fragments from pAJ582 into the BglII and HindIII sites of pAJ1315. pAJ1278 (*GAL10::myc-LSG1[K349T]*) and pAJ1312 (*GAL10::myc-LSG1*) were each generated by ligating the BamHI/HindIII fragments of pAJ1109 and pAJ879, respectively, into BamHI/HindIII sites of pRS316.

Indirect immunofluorescence. Indirect immunofluorescence was performed as described previously (Ho et al., 2000b). Antibodies used were monoclonal α-c-myc (9E10; Covance) for the primary antibody (1:1000 dilution), and the secondary antibody was Cy2-conjugated α-mouse antibody (1:300 dilution; Jackson IRL). Fluorescence was visualized on a Zeiss Axiophot microscope fitted with a X100 objective lens and a Princeton Electronics Micro-MAX charge-coupled device camera controlled with the IPLab Spectrum P software package from Signal Analytics Corp or on a Nikon E800 microscope fitted with a X100 objective and a Diagnostic Instruments SPOT II camera controlled with SPOT software. Images were prepared using Adobe Photoshop 5.0.

GFP Fluorescence. GFP visualization was adapted from a method described previously (Stage-Zimmermann et al., 2000). Culture conditions are given in corresponding figure legends. Cells were fixed with 0.11 volumes of 37% formaldehyde for 40min. The cells were washed in 0.1M potassium phosphate pH 6.6 and resuspended in Ksorb buffer (0.1M potassium phosphate pH6.6, 1.2M sorbitol). 0.05% Triton X-100 was added to permeabilize cells for 4min. and 1µg/ml DAPI was added to stain nuclei. After 2min, cells were washed twice and resuspended in PBS and visualized as described for indirect immunofluorescence.

LMB treatment. For *RPL10* experiments, overnight cultures in galactose were diluted to OD_{600}~0.1 and incubated for 4hrs at 30°C. Cultures were divided in half and 2% glucose was added to one half while the other was left in galactose. Cultures were incubated 4 h more before being concentrated 10-fold in fresh medium. For *LSG1* experiments, overnight cultures were diluted to OD_{600}~0.1 into fresh medium and re-cultured until OD_{600}~0.2. Cells were then supplemented with galactose (1%) and cultured for an

additional 3 h (final OD_{600} ~ 0.4). Cells were then concentrated 20-fold in fresh medium. For both preparations, LeptomycinB (LMB) (M. Yoshida) was then added at a final concentration of 0.1µg/ml and cultures were incubated an additional 20 min before fixation and visualization as described above.

Immunoprecipitations. Co-immunoprecipitations of Nmd3-myc proteins were carried out as follows. All steps were carried out on ice or at 4°C. Cells were washed in lysis buffer (20mM Tris-HCl pH7.5, 150mM NaCl, 10% glycerol, 0.1% NP40, 1mM $MgCl_2$ and protease inhibitors) and pelleted. The cells were resuspended in one volume of lysis buffer, and extracts were made by glass bead lysis (5x50sec with 1min intervals on ice). Insoluble material was pelleted at 15000xg for 10min at 4°C. 1.5 µl of α-c-myc (9E10; Covance) antibody was added to equal OD_{260} units of sample supernatants and rocked for 1hr at 4°C. 30µl of BSA-blocked protein A agarose beads (Invitrogen) were then added, and rocking was continued for an additional 1hr. Beads were washed 3x with lysis buffer and eluted in 50µl of 1x SDS-PAGE sample buffer without β-mercaptoethanol. Proteins were run on a 12% SDS-PAGE gel and transferred to nitrocellulose. Blots were blocked in non-fat milk and probed with α-c-myc or α-Rpl8p (this work) as indicated in Figure 6B.

Polysome Analysis. Sucrose density gradient fractionation and polysome analysis were essentially performed as described previously (Ho and Johnson, 1999) from cultures treated and collected as indicated in respective figure legends. Nine A_{260} units were loaded onto each gradient. Proteins were precipitated from gradient fractions with trichloroacetic acid, separated on12% SDS-PAGE gels and western blotting performed as

for immunoprecipitations with either α-Nmd3p or α-Rpl1ap (F. Lacroute) as indicated in Figure 3D.

Antibodies

Rabbit polyclonal anti-Nmd3p was raised against GST-Nmd3p expressed and purified from yeast (Ho et al., 2000a) and affinity purified using denatured HIS6-tagged Nmd3p expressed in bacteria. Rabbit polyclonal Rpl8p was raised and affinity purified against HIS6-tagged Rpl8p expressed in *E. coli*.