Supporting Information

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SI Methods

siRNA Screen. SMART pools (Dharmacon), comprising four duplexes targeting a single human mRNA transcript, were individually arrayed into wells of black, clear-bottom 384-well plates (Costar 3712; Corning) containing a 1:100 dilution of Lipofectamine 2000 (Invitrogen) in OptiMEM (Invitrogen). Duplexes and lipids were incubated for 20 min at room temperature and mixed with HeLa cells to yield final concentrations of 5×10^4 cells/mL and 50 nM siRNA. Plates were inoculated with 1,250 HeLa cells per well, and cells were centrifuged for 5 min at $700 \times g$. At 48 h after transfection, the ~5,000 cells were inoculated with 25,000 infectious particles of rVSV-eGFP. Cells were fixed 7 h later with 2% formaldehyde in PBS, the nuclei were counterstained with 4 μg/mL Hoechst nuclear dye (33342; Invitrogen) for 10 min at room temperature, and unincorporated dye was removed by washing once with 60 μL PBS per well. Individual wells were examined using a cellWoRX High Content Cell Analysis System (Applied Precision), and the cell-scoring module of MetaXpress Software (Molecular Devices) was used to quantify the total number of cells and percentage of EGFP-positive cells. All samples were performed in duplicate.

Cells and Viruses. HeLa cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS (Tissue Culture Biologicals). Vesicular stomatitis virus (VSV), rVSV-EGFP, rVSV-M51R, and rVSV-Luc were amplified in BHK-21 cells (ATCC), purified through a 10% sucrose (wt/vol) cushion prepared in NTE (10 mM Tris-pH 7.4, 100 mM NaCl, 1 mM EDTA), and virus stocks were stored in NTE at −80 °C (1). The 5′ and 3′ UTR sequences of the VSV luciferase mRNA were AACAGTAATCAGAATTCTCGAGAAAGCCACC (genomic coordinates: 51–81) and TGGCCATATGAAAAAAA (genomic coordinates: 1735–1751), respectively. Other viruses were kind gifts: poliovirus (J. Hogle, Harvard Medical School, Boston, MA), measles virus-GFP (P. Duprex, Boston University, Boston, MA), NDV-GFP (A. Garcia-Sastre, Mount Sinai School of Medicine, New York, NY), and RABV-mCherry (E. Calloway, Salk Institute, La Jolla, CA).

Microscopy. Cells were fixed with 2% paraformaldehyde for 15 min, washed with PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4), and where indicated, stained with fluorescent DNA binding stain, DAPI. Coverslips were mounted using ProLong Antifade (Invitrogen). For high-magnification images (63×, 20×), cells were imaged on a Zeiss Axioplan 2 inverted fluorescence microscope (Carl Zeiss MicroImaging) with a Hamamatsu Orca-HR (C4742-94) camera (Hamamatsu) and analyzed using Axiovision software. For low-magnification images (10×), cells were imaged on the cellWoRx high-content screening microscope.

Plasmids, siRNAs, and Transfection. The pFR-CrPV (Cricket paralysis virus) bicistronic luciferase reporter plasmid was obtained from P. Sharp through Addgene (plasmid 11509). The plasmid pRL-CMV (Promega) was used to generate renilla luciferase mRNA in HeLa cells for in vitro translation. To generate the rpL40-expressing plasmid (pcDNA3.1-rpL40), rpL40 was amplified from a SP73 vector containing rpL40 (a kind gift from K. Hertel, University of California, Irvine, CA) and inserted into the Acc65I and XhoI sites of pcDNA3.1 (Invitrogen). siRNA-resistant pcDNA3.1-rpL40 was generated by site-directed mutagenesis using the QuikChange methodology (Stratagene) and primers rpL40-SM-F (5′-GGTGTTGCGCCTGCGAGGTGGGATCATCGAGCCTTCTCTCCGCCAGC-3′) and rpL40-SM-R (5′-GCTGGCGGAGAGAAGGCTCGATGATCCCACCTCGCAGGCGCAACACC-3′). Identical cloning strategies were used for constructing expression vectors for CLG1 (YGL215W) and DDR2 (YOL052C-A). Briefly, the 5′ UTR was cloned into pGEM3 using the EcoRI and BamHI sites; luciferase and the 3′ UTR were double ligated into the vector using the BamHI, NotI, and SalI sites; and two copies of a T7 terminator were added using the SalI and HindIII sites.

The 5′ and 3′ UTR sequences of the DDR2 reporter mRNA were AAGCAAGCACGCTAATTTAATATCGATTTAAAC and GAAAAACGCCGCTTACTGCCACGATGATGATACCCTATTGACGTTTCTGAAATGTATAATTTCTTTTCTCATCTTCCCCTTTGATATTCCATCTATAGGCCCCAGAGTAGTAAATTTGGTGCTTTAATTTTTTTTCTCTTCTTCCAGTTCGTCTCTATTTTTTCATTCCATTATATTTATTTATCAGTTTTACTTTCTCAAATATTCTTATATAACACTATTTCATCTACGTAACCGAAAATAAG, respectively (2). The 5′ and 3′ UTR sequences of the CLG1 reporter mRNA were ACAAATCATTGATCTTTTAAGAAAAACGCACAAGGATCATATACTAGATTCTCGTTCTCGTTTTTTCTTCCTTTTTTTTTTCTCTAAAGCCTTTTACTGGGTTAATTTCCTTTATTGACCCAAATTAAAAGAAAACGTTTCTCAGGAGACTCTTTTAAGCAAAATTTAGCAAATTTGTGTTTGCTGTTGTTTTTACAGAGACTGCATTACTTGAAGGTTTGCCTTTAAGTCTTCGAGTCGTTTTTTTTTATTAACTTTAATCCTTTTTCTGTGTGTTTGTGTATATTCAGTGGGGTTATTTTACAGTATTCGAAGAGACTTCGTTTCACACATTTAAACCAGCTTTATTAGCGTTTAGCTTATACACTACAAGGAATTTTTTTTTTCTTTAACATTATTAAGACAGTTATTGAGTTAATTCGTCTTCAGCCCCCCTCCCCCCAACAAACCCCCCTTCATATAGAA and TGAAAACTTTTTTTTCCTTATTTTTTCTTTGATCCCATCGAATTACTTTCTCTTTTGCCCAGGATCATCTTTCTAATCTATCATTTTATTATTTTCTCTCATGAGAAAACAGAATTTCGAAACAGATATAATAAAAAAATTGAAAAATTTGAAAAAAAAAAATCTAGAAACATATTTTCCTAAAATGAAAATCGGAAGCTAACAAAATTTTTGAAAAACGAAATAAAGAAGAAAGATTATTATTATTACTTTTTTTATTAGTACTCCATATGGACCTCTTAGGTGAGTGATCTTATT, respectively (2).

Plasmid transfections were performed using Lipofectamine 2000 according to the manufacturer's instructions. For rescue of the siRNA-induced phenotype, HeLa cells were transfected with the indicated plasmid 24 h before transfection of cells with siRNA. Transfection of plasmids into vTF7-3–infected bsrT7 cells was performed as described, except using 12 μg of plasmid per 10-cm dish, with cells at 80% confluency (1). RNA was harvested and purified from cells at 24 h after transfection.

Individual siRNAs used were siGENOME nontargeting siRNA 3 (D-001210-03), rpL40-1 (D-011794-02), rpL40-2 (D-011794-04), and rpL22 (D-011143-02) (Dharmacon). For siRNA transfection into a 24-well plate, 1 μL of Lipofectamine 2000 was mixed with 100 μL of OptiMEM and incubated at room temperature for 5 min. One hundred microliters of OptiMEM and 1.5 μL of 20 μM siRNA were added, mixed, and incubated at room temperature for 15 min. Trypsinized HeLa cells (3×10^4) in 400 μL of DMEM with 10% FBS was added to the lipid-siRNA mix and plated, and experiments were performed 48 h later. This protocol was scaled accordingly to different cell culture surface areas.

Ribonucleoprotein Transfection. To isolate ribonucleoproteins (RNPs), purified VSV was mixed with 12.5 mM Tris, pH 7.4, 5% glycerol, 5 mM EDTA, pH 8, 3.5 mM DTT, 0.1% Triton X-100, and 500 mM CsCl in a total volume of 600 μL and incubated on

ice for 1.25 h. Six hundred microliters of 20 mM Tris, pH 7.4, and 3.5 mM DTT were added and loaded onto a 30–50% (vol/vol) glycerol gradient made in NTE with 3.5 mM DTT. Gradients were spun for 3.5 h at 4 °C at 45,000 rpm in a SW50.1 rotor, and the pellet was resuspended in NTE. rVSV-Luc RNPs (0.4 μg) were transfected into HeLa cells using Lipofectamine 2000, and luciferase was assayed at 7 h after transfection.

RNA Analysis. For metabolic labeling of RNA, cells were labeled with 33 μCi/mL [^{3}H]-uridine (Perkin-Elmer). Cells were lysed in rose lysis buffer (1%, vol/vol Nonidet P-40 alternative; 0.4%, vol/vol sodium deoxycholate; 66 mM EDTA; 10 mM Tris, pH 7.4), and cytoplasmic RNA was purified by phenol/chloroform extraction and analyzed by electrophoresis on an acid-agarose gel. For analysis of primary transcription, cells were treated with 100 μg/mL cyclohexmide for 30 min before infection. Cells were infected for 5 h at a multiplicity of infection (MOI) of 100, and total cytoplasmic RNA was isolated by phenol chloroform extraction and analyzed by quantitative real-time PCR. For analysis of total RNA synthesis, cells were infected at an MOI of 5 for 5 h, and RNA was analyzed by metabolic labeling.

Quantitative Real-Time PCR. cDNA was reverse-transcribed from RNA isolated from mock-infected or infected cells using random hexamers or VSV-*N*-RT-dT primer (5′-TTTTTTTTTTTTTTTT-CATATGTAGC-3′), respectively, and SuperScript III (Invitrogen), following the manufacturer's instructions. Real-time PCR was performed using *Power* SYBR Green PCR Master Mix and a Prism 7300 sequence detection system (Applied Biosystems) according to the manufacturer's instructions. The final reaction volume was 20 μL, containing 2 μL cDNA and 100 nM of each primer. Primers VSV-*N*-F (5′-GCAAATGAGGATCCAGTGG-3′) and VSV-*N*-R (5′-CAGGGCTTTCAAGGATAC-3′) were used to detect VSV N cRNA. Primers β-actin-qRTPCR-F (5′-TCCCTGGAGAAGA-GCTACG-3′) and β-actin-qRTPCR-R (5′-GTAGTTTCGTG-GATGCCACA-3′) were used to amplify β-actin cDNA. To ensure specificity of each primer pair, a dissociation curve of the PCR products was determined. Samples were run in duplicate, and relative copy numbers were determined from a standard curve generated by serial dilutions of a plasmid containing VSV N or cDNA reverse-transcribed from total HeLa RNA.

Protein Analysis. For metabolic labeling of proteins, cells were starved of L-methionine and L-cysteine for 30 min. Proteins were labeled for 2 h by addition of 25 μCi/mL [^{35}S] EasyTag express (Perkin-Elmer). Cells were lysed in rose lysis buffer (1%, vol/vol Nonidet P-40 alternative; 0.4%, vol/vol sodium deoxycholate; 66 mM EDTA; 10 mM Tris, pH 7.4), and equal amounts of total cytoplasmic protein were analyzed on a low-bis 10% polyacrylamide gel and detected using a phosphoimager. Western blot analysis was performed using anti-actin (1:5,000; Chemicon) or anti-rpL40 (kind gift from K. Redman, Indiana University, Fort Wayne, IN) antibodies.

Sequencing Analysis. Sequencing reads were aligned against the Ensembl EF3 *Saccharomyces cerevisiae* genome. First, residual noncoding RNAs were filtered out using Bowtie, and then the remaining sequences were aligned using Tophat, allowing up to two mismatches (3, 4). Reads were assembled, and abundance was measured using Cufflinks. mRNA abundance was measured by calculating reads per kilobase of exon per million fragments mapped (RPKM), allowing us to take into account differences in total reads and gene lengths. Genes were filtered to require at least 256 reads for the rpL40-containing samples. To form our list of candidate mRNAs (fold reduction > 3), we disregarded transcripts that were likely identified due to off-target effects of changing the carbon source and ribosome biogenesis feedback loops. Genes were annotated with functional descriptions using the *Saccharomyces* Genome Database.

1. Whelan SP, Ball LA, Barr JN, Wertz GT (1995) Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones. *Proc Natl Acad Sci USA* 92(18):8388–8392.
2. Nagalakshmi U, et al. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* 320(5881):1344–1349.
3. Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10(3):R25.
4. Trapnell C, Pachter L, Salzberg SL (2009) TopHat: Discovering splice junctions with RNA-Seq. *Bioinformatics* 25(9):1105–1111.

Fig. S1. RpL40 is required for VSV replication. (*A*) VSV gene expression following rpL40 siRNA treatment. HeLa cells were reverse transfected with a control nontargeting siRNA (NT) or one of two rpL40-targeting siRNAs (rpL40-1 and -2) and seeded onto coverslips. At 48 h posttransfection, cells were infected with rVSV-eGFP at a MOI of 1, fixed at 6 hpi, and examined by epifluorescence microscopy. (*B*) Analysis of rpL40 mRNA levels by RT-PCR. To control for equal RNA levels in the cell extracts, RT-PCR to detect the proteosomal transcript proteasome subunit β type 6 (PSMB6) was also performed. (*C*) Analysis of rpL40 protein levels by Western blot. As a control for equal protein levels, an antibody was used to detect actin. (*D*) VSV production on rpL40 knockdown. siRNA-treated cells were infected with VSV, and output titers were measured by plaque assay at indicated times after infection.

Fig. S2. Sensitivity of VSV to rpL40 depletion does not reflect defects in ribosome biogenesis and maturation. (*A*) Accumulation of mature ribosomal RNAs in cells treated with rpL40 siRNA. RNA was labeled with [^{3}H]-uridine for 2 h. Cytoplasmic RNA was purified by phenol/chloroform extraction and analyzed by electrophoresis on an acid-agarose gel. (*B*) Accumulation of mature ribosomal RNAs in rVSV-M51R–infected cells. Cells were mock infected or infected at an MOI of 1 with VSV or rVSV-M51R. At 3 hpi, RNA was labeled and analyzed as in *A*. (*C*) rVSV-M51R protein synthesis in siRNA-transfected cells. Cells were infected at an MOI of 1 and exposed at 6 hpi to [^{35}S] methionine-cysteine in the presence of actinomycin D [10 μg/mL] for 30 min. (*D*) VSV gene expression following rpL22 siRNA treatment. HeLa cells reverse transfected with a control nontargeting siRNA (NT) or a rpL22-targeting siRNA were infected with rVSV-eGFP at a MOI of 1, fixed at 6 hpi, and examined by epifluorescence microscopy. (*E*) Analysis of rpL22 mRNA levels by RT-PCR. RT-PCR using PSMB6 primers was performed as a control for equal RNA levels in the cell extracts.

Fig. S3. Reproducibility of RPKM between biological replicates. (*A*) Lysates from yeast cells depleted of rpL40 (glucose) or not depleted of rpL40 (galactose) for 4 h were resolved by sucrose gradient centrifugation. Extracts were prepared twice for biological replicates, and RNA from polysome-containing fractions, as indicated, was isolated to make libraries for deep sequencing. (*B*) Comparison in mRNA density for biological replicates. mRNA density is expressed as RPKM to account for differences in gene lengths and total reads. (*C*) Error distribution of gene replicates. Normal error curve and histogram of log_2 ratios between replicates for genes that have more than 256 reads in rpL40-containing samples are plotted.

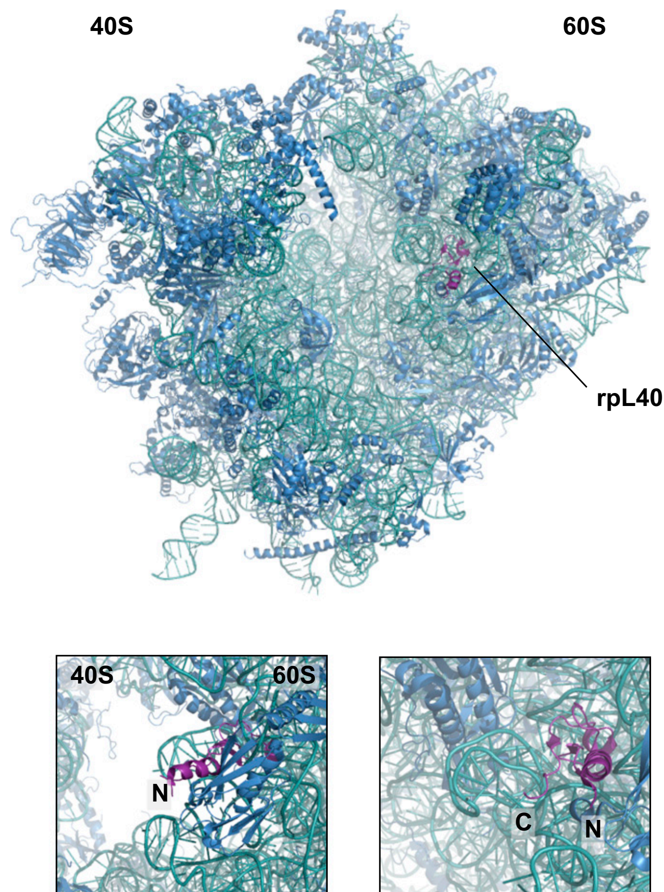

Fig. S4. Localization of rpL40 on the 80S ribosome. Localization of rpL40 on the crystal structure of the 80S ribosome. Ribosomal proteins are blue, and ribosomal RNAs are teal. RpL40 is magenta (PDB 2XZM, 4A17, 3A19) (1). N and C termini are indicated in the two rotated views of rpL40.

1. Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N (2011) Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6. *Science* 334(6058): 941–948.

Organism	Sequence
Archaea (*S. solfataricus*)	MPLTDPAKLQIVQQRVFLKKVCRKCGALNPIRATKCRR--C-HSTNLRLKKKELPTKKG
Flatworm (*S. japonicum*)	--IIEPTLKALAQKYNCEKMICRKCYARLHPKATNCRKRKCGHTSNVRPKKKLR-----
Yeast (*S. cerevisiae*)	--IIEPSLKALASKYNCDKSVCRKCYARLPPRATNCRKRKCGHTNQLRPKKKLK-----
Green algae (*C. reinhardtii*)	--IIEPSLQALARKYNQEKMICRKCYARLHPRAKNCRKKSCGHTNQLRPKKKLK-----
Segmented worm (*E. complanata*)	--IIEPSLRMLAQKYNCDKMICRKCYARLHPRATNCRKKKCGRTSNIRPKKKIK-----
Human (*H. sapien*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Monkey (*M. fascicularis*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Cow (*B. taurus*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Pig (*S. scrofa*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Mouse (*M. musculus*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Cat (*F. catus*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Giant panda (*A. melanoleuca*)	--IIEPSLRQLAQKYNCDKMICRKCYARLHPRAVNCRKKKCGHTNNLRPKKKVK-----
Fly (*D. melanogaster*)	--IIEPSLRILAQKYNCDKMICRKCYARLHPRATNCRKKKCGHTNNLRPKKKLK-----

Fig. S5. Alignment of rpL40 sequences. RpL40 sequences, with the C-terminal ubiquitin extension removed, were aligned using MUSCLE and visualized using Jalview. Colored sequences indicate more than 70% conserved identity. GenBank IDs are *Homo sapien* (AAI01833.1), *Saccharomyces cerevisiae* (CAA86130.1), *Macaca fascicularis* (P0C273.2), *Bos taurus* (DAA28295.1), *Sus scrofa* (NP_999376.1), *Mus musculus* (NP_063936.1), *Felis "koz" catus* (NP_001116826), *Ailuropoda melanoleuca* (AEA39530.1), *Drosophila melanogaster* (NP_476776.1), *Eurythoe complanata* (ABW23236), *Schistosoma japonicum* (226477078), *Chlamydomonas reinhardtii* (EDO98280.1), and *Sulfolobus solfataricus* (Q980V5.1).

Table S1. SiRNA screen of ribosomal proteins required for VSV replication. Ribosomal proteins are grouped by phenotype of knockdown as indicated

Table S1

Table S2. List of cellular transcripts whose polysome association was reduced by more than threefold upon depletion of rpL40

Table S2