Supporting Information

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SI Materials and Methods

Cell Culture and siRNA Transfection. HeLa cells (ATCC) were grown in Minimum Essential Medium supplemented with 10% FBS, glutamine, and nonessential amino acids at 37 °C with 5% CO_2. For siRNA experiments, three siRNA duplexes were used for fibrillarin silencing: 5′-GUCUUCAUUUGUCGAGGAA-AdTdT-3′; 5′-UGGAGGACACUUUGUGAUUUUdtdT-3′; and 5′-CUGUCAGGAUUGCGAGAGAdTdT-3′.

The control siRNA does not target any human sequence (negative control siRNA duplex; Eurogentec). HeLa cells were transfected using the X-tremeGENE siRNA reagent (Roche) according to the manufacturer's instructions. Seventy-two hours after siRNA transfection, cells were plated according to future analyses.

Transfection and Plasmid Construction. For siRNA experiments, plasmids containing the bicistronic IRES luciferase constructs were described previously (7, 41).

Dual Luciferase Assays for in Cellulo Translation Assays. pIRES-FGF1, pIRES-EMCV, and pIRES-VEGFA were donated by A. C. Prats, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France and pRF-CrPV was a gift from D. Ruggero, University of California, San Francisco. Luciferase assays were performed 24 h after transfection with the reporter plasmids using X-tremeGENE 9 reagent (Roche). Dual luciferase assays were performed using the Dual-Glo luciferase reagent (Promega) according to the manufacturer's instructions, and a Tecan M1000 plate reader. IRES translation initiation is the ratio of background subtracted signal of Firefly luciferase over *Renilla* luciferase.

Global Protein Synthesis. Global protein synthesis analysis by puromycylation followed by puromycin detection was performed essentially as in ref. 31. Puromycin incorporation was detected by Western blot on whole-cell protein extracts. Global protein synthesis by isotope labeling was performed as previously described (46) by incubating cells for 30 min, with a [^{35}S]-methionine–[^{35}S]cysteine mix. Proteins were separated by SDS/PAGE on a 4–15% gradient polyacrylamide gel (Bio-Rad).

Real-time quantitative RT-PCR. Two-hundred nanograms of total RNA were reverse transcribed using the MMLV RT kit and random primers (Invitrogen), according to the manufacturer's instructions. Quantitative real-time PCR (RT-qPCR) was carried out using the Light cycler 480 II real-time PCR thermocycler (Roche). Expression of mRNAs was quantified using LightCycler 480 SYBR Green I Master Mix (Roche) and normalized using HPRT1 expression according to the 2-ΔΔCt method.

Primers were as follows: LucR-Fwd 5′-AACGCGGCCTCT-TCTTATTT, LucR-Rev 5′-ACCAGATTTGCCTGATTTGC, LucF-Fwd 5′-AACACCCCAACATCTTCGAC, LucF-Rev 5′-TTTTCCGTCATCGTCTTTCC, HPRT1-Fwd 5′-TGACACTG-GCAAAACAATGCA, HPRT1-Rev 5′-GGTCCTTTTCACCAG-CAAGCT.

Immunofluorescence analysis. Cells were grown on glass coverslips, fixed in 4% of paraformaldehyde in PBS before permeabilization with 0.5% Triton X-100 in PBS. Fibrillarin and nucleolin were detected using the anti-FBL rabbit polyclonal antibody (ab5821; Abcam) diluted at 1:2,000, and the anti-NCL mouse monoclonal antibody (ab13541; Abcam) at 1:4,000. Secondary antibodies were labeled with AlexaFluor488 or AlexaFluor555 (Molecular Probes), and used at 1:1,000. Coverslips were mounted using the Fluoromount G mounting medium (EMS). Images were acquired on a Nikon NiE fluorescence microscope using a 60× Plan Apochromat immersion objective (NA 1.4) and a Flash 4.0 CMOS camera (Hammamatsu).

Ribosome purification. Ribosomes were purified as previously described (24). Briefly, cytoplasmic fractions were obtained by mechanical lysis of cells with a Dounce and centrifugation at 12,000 × *g* for 10 min to pellet mitochondria. Cytoplasmic fractions were loaded onto a 1 M sucrose cushion in a buffer containing 50 mM Tris·HCl pH 7.4, 5 mM $MgCl_2$, 500 mM KCl, and 2 mM DTT, and centrifuged for 2 h at 240,000 × *g*. The pellet containing the ribosomes was suspended in a buffer containing 50 mM Tris·HCl pH 7.4, 5 mM $MgCl_2$, and 25 mM KCl.

Ribosome Production. Ribosome production was measured as described previously (24). Briefly, cells were incubated for 1 h in methionine-cysteine-free DMEM supplemented with a [^{35}S]-methionine–[^{35}S]cysteine mix (GE Healthcare). Incorporation of radioactive amino acids was measured from one unit ($OD_{260\ nm}$).

Two-dimensional gel electrophoresis. For 2D gel electrophoresis, 5 $OD_{260\ nm}$ units of ribosomes extracted from [^{35}S]-methionine–[^{35}S]cysteine-labeled cells were used. The method was previously described (24). Briefly, ribosomal proteins were extracted using acetic acid, extensively dialyzed against 1 M acetic acid and lyophilized. After lyophilizing, proteins were solubilized and reduced in 6 M guanidine hydrochloride, 0.5 M Tris·HCl pH 8.5 and 10 mM DTE. Proteins were then alkylated by adding 40 mM iodoacetamide in 6 M guanidine hydrochloride and 0.5 M Tris-HCl pH 8.5. Proteins were lyophilized and solubilized in sample buffer (20 mM Tris-boric acid pH 8.3, 1 mM EDTA and 8 M urea).

In the first dimension, proteins were separated according to their electric charge, in 4% polyacrylamide gels containing 0.2 M Tris-boric acid pH 8.6, 8 M urea, and 10 mM EDTA, placed in glass tubes. At the end of the first dimensional run, gels were extracted from the tube and equilibrated for 5 min in 0.625 M Tris·HCl pH 6.8, 2% SDS, and 0.002% Bromophenol blue. For the second dimension, the gels from the first dimension were placed in a 1.5-mm-thick gel cast made of 14% polyacrylamide (37.5:1 acrylamide: *N*-*N*′methylenebisacrylamide). Protein separation was achieved using standard SDS/PAGE conditions. Proteins were stained by 0.1% Coomassie brilliant blue R 250. Gels were dried and exposed to PhosphoImaging screen. All chemicals were purchased from Sigma-Aldrich.

Mass spectrometry-based proteomic analyses of purified ribosomes. Label-free quantitative proteomics has been performed as in Casabona et al. (38). Ribosomes purified at 500 mM KCl were solubilized in Laemmli buffer, stacked in the top of a 4–12% NuPAGE gel (Invitrogen), and stained by R-250 Coomassie blue (Bio-Rad). Gel bands were excised and in-gel proteins were digested using trypsin (Promega). Resulting peptides were analyzed by nanoliquid chromatography coupled to tandem mass spectrometry (Ultimate 3000 coupled to LTQ-Orbitrap Velos Pro; Thermo Scientific) using a 120-min gradient. RAW files were processed using MaxQuant v1.5.3.30. Spectra were searched against the SwissProt database (*Homo sapiens* taxonomy, October 2016 version) and the frequently observed contaminants database embedded in MaxQuant. Trypsin was chosen as the enzyme and two missed cleavages were allowed. Precursor mass error tolerances were set respectively at 20 ppm and 4.5 ppm for first and main searches. Fragment mass error tolerance was set to 0.5 Da. Peptide modifications allowed during

the search were: carbamidomethylation (C, fixed), acetyl (Protein N-ter, variable) and oxidation (M, variable). Minimum peptide length was set at seven amino acids. Minimum number of peptides, razor + unique peptides and unique peptides were all set at 1. Maximum false-discovery rates (FDR)—calculated by employing a reverse database strategy—were set at 0.01 at peptide and protein levels. iBAQ values were calculated from MS intensities of unique + razor peptides and used for statistical analyses using ProStar. Proteins identified in the reverse and contaminant databases or exhibiting less than 5 iBAQ values in one condition were discarded from the matrix. After $\log_2$ transformation, iBAQ values were median-normalized, missing data imputation was carried out (replacing missing values by the 2.5-percentile value of each column) and statistical testing was conducted using the *limma t* test. Differentially expressed proteins were sorted out using a $\log_2$ (fold-change) cut-off of 2, and a FDR threshold on *P* values of 1% using the Benjamini–Hochberg method.

For generation of Fig. 1*C*, iBAQ values from ribosomal proteins were sorted out before column-wise normalization; to facilitate representation (data centering on 1), for each ribosomal protein the normalized iBAQ values were divided by the mean of the 10 values obtained.

Western blot analysis. Twenty micrograms of total protein lysates were run on a 12% SDS polyacrylamide gel and transferred onto a nitrocellulose membrane. The membrane was blocked with 3% nonfat milk in TBST. The antibodies used were the following: fibrillarin (ab166630; Abcam) at 1:2,000, Dyskerin (sc-48794; Santa Cruz) at 1:500, nucleophosmin (sc-5564; Santa Cruz) at 1:500, puromycin (clone 12D10; EMD Millipore) at 1:4,000, and Ku80 (ab3715; Abcam) at 1:2,000. Antibodies were incubated for 1 h in 1% milk-TBST. Proteins were detected by chemiluminescence using an anti-rabbit or anti-mouse peroxidase-conjugated antibody (Cell Signaling) diluted 1:10,000, and Clarity ECL substrate (Bio-Rad). Images were collected on a ChemiDoc XRS+ (Bio-Rad) and the signal analyzed using the Bio-Rad ImageLab software.

Northern blot analysis. Northern blot was performed as described in Belin et al. (24). The probes were obtained by oligonucleotide synthesis (Eurogenetec): ETS1-1399–5′-CGCTAGAGAAGGCTTTTCTC-3′; ITS1-5′-CCTCTTCGGGGGACGCGCGCGTGGCCCCGA-3′; and ITS2-5′-GCGCGACGGCGGACGACACCGCGGCGTC-3′.

Next, 50 pmoles of each oligonucleotide probe was labeled in the presence of 50 pmoles of [γ-^{32}P] ATP (Perkin-Elmer) and T4 polynucleotide kinase (New England Biolabs) for 30 min at 37 °C.

Three micrograms of nuclear RNAs were resolved on a 1% denaturing agarose gel and blotted onto a Hybond-N+ membrane (GE Healthcare). Signal detection was performed using a PhosphorImager (FLA 9500; GE Healthcare). Total 28S and 18S rRNA were visualized by fluorescence imaging following ethidium bromide staining, and were used as loading controls.

Analysis of rRNA methylation by isotope labeling. Cells were incubated for 1 h in DMEM supplemented with 10% heat-inactivated dialyzed FCS and [^{3}H]-methyl-methionine at a final concentration of 15 μCi/mL (GE Healthcare). After 1-h labeling, cells were washed three times with ice-cold PBS, and scrapped. Nuclei were isolated by mechanical fractionation and nuclear RNAs were extracted and separated on formaldehyde agarose gels. RNAs were transferred onto nitrocellulose membranes. Radioactivity of the 45S/47S pre-rRNA was measured after exposure on a PhosphorImager screen, using a Typhoon scanner (GE Healthcare).

Analysis of rRNA methylation by RiboMethSeq. RiboMethSeq is based on protection of phosphodiester bonds against alkaline hydrolysis conferred by replacement of the 2′OH by a methyl group. The 3′-downstream nucleotide is thus absent at the 5′-end in the collection of RNA fragments. Partial alkaline hydrolysis of total RNA samples is followed by deep-sequencing and allows 5′-end counting for every fragment. The calculated MethScore represents the level of methylation of a given nucleotide in the ribosomal population.

RiboMethSeq was performed essentially as described previously (9), and is presented in detail below.

RNA fragmentation. RNA (1–250 ng) was subjected to alkaline hydrolysis in 50 mM bicarbonate buffer pH 9.2 for 4–10 min at 95 °C. The reaction was stopped by ethanol precipitation using 0.3 M Na-OAc pH 5.2 and glycoblue as a carrier in liquid nitrogen. After centrifugation, the pellet was washed with 80% ethanol and resuspended in nuclease-free water. The size of RNA fragments (30–200 nt) generated was assessed by capillary electrophoresis using a PicoRNA chip on Bioanalyzer 2100 (Agilent).

End repair of RNA fragments. Fragmented RNA without an additional fractionation step was 3′-end dephosphorylated using 5 U of Antartic Phosphatase (New England Biolabs) for 30 min at 37 °C and after inactivation of the phosphatase for 5 min at 70 °C, RNA was phosphorylated at the 5′-end using T4 PNK and 1 mM ATP for 1 h at 37 °C. End-repaired RNA was then purified using RNeasy MinElute Clean-up kit (Qiagen) according to the manufacturer's recommendations, except that 675 μL of 96% ethanol were used for RNA binding. Elution was performed in 10 μL of nuclease-free H_2O.

Library preparation. RNA was converted to a library using NEBNext Small RNA Library kit (New England Biolabs) following the manufacturer's instructions. Library quality was assessed using a High Sensivity DNA chip on a Bioanalyzer 2100. Library quantification was done using a fluorometer (Qubit 2.0 fluorometer; Invitrogen).

Sequencing. Libraries were multiplexed and subjected to high-throughput sequencing using an Illumina HiSEq. 1000 instrument with 50-bp single-end read runs. Since clustering of the short fragments was very efficient, libraries were loaded at a concentration of 6–8 pM per lane.

Bioinformatics pipeline. Adapter sequence trimming was done using Trimmomatic-0.32 with the following parameters: LEADING:30 TRAILING:30 SLIDINGWINDOW:4:15 MINLEN:17 AVGQUAL:30. Alignment to the reference rRNA sequence was done by Bowtie2 (v2.2.4) in End-to-End mode. The 5′-end counting was done directly on *.sam file using dedicated Unix script. Final analysis was performed by calculation of MethScore for quantification of 2′-O-Me residues.

Ribosome Structure Analysis. Methylated nucleotides were mapped on the cryo-EM structure of the human ribosome (PDB ID code 4UG0) (30). Reference structure of prokaryotic ribosome containing A-, P-, and E-site tRNAs plus mRNA was from *Thermus thermophilus* (PDB ID code 4V5C) (39). Observations of methylation site positions with regards to active sites of the ribosome were made after sequence alignment and structural superposition of the 23S rRNA of the *T. thermophilus* ribosome onto the 28S rRNA of human ribosome for the PTC and the CCA-end binding pocket of the E-site or sequence alignment and structural superposition of the 16S rRNA of the *T. thermophilus* ribosome onto the 18S rRNA of human ribosome for the decoding center and mRNA path. Figures and sequence alignment followed by structure superposition were performed using PyMOL 1.4 (Schrödinger; https://pymol.org/2/).

shRNA-Expressing Cells Lines. HeLa cell lines expressing an inducible shRNA were generated by lentiviral infection. Lentiviral particles were produced using the pTRIPZ-shRNA-NS and pTRIPZ-shRNA-FBL-351067 vectors, which were acquired from Open Biosystems (Dharmacon). Upon lentiviral infection, cell populations were selected using puromycin at 2 μg/mL for 14 d. Cells were grown under selection at 1 μg/mL and cultured without puromycin 24 h before any experiment. shRNA expression was induced by doxycycline treatment for 5 d at 1 μg/mL. Induction efficiency was monitored by evaluating the tRFP expression

marker, by observation under fluorescent microscope or by flow cytometry. Change in FBL levels was verified by Western blotting.

Ribosome profiling.

Ribosome protected fragments preparation and sequencing. HeLa ± FBL-shRNA cells were cultivated during 5 d with 1 μg/mL doxycycline. Next, 150 million cells were treated with 100 μg/mL cycloheximide for 10 min at 37 °C. Then, cells were suspended in lysis buffer (10 mM Tris-PO_4 pH7.5, 100 mM KCl, 10 mM Mg^{2+} acetate, 1% Triton X-100, 2 mM DTT, 100 μg/mL cycloheximide). Glass beads were added and cells were lysed by vortexing 5 min at 4 °C. The supernatant was collected and digested with 15 U RNase I AMBION/OD_{260} for 1 h at 25 °C. To purify monosomes, digested extracts were loaded on a 24% sucrose cushion (50 mM Tris-acetate pH 7.6, 50 mM NH_4Cl, 12 mM $MgCl_2$, 1 mM DTT) and centrifuged at 413,000 × *g* for 2 h 15 min. Pellets were washed with lysis buffer and suspended in the same buffer. Ribosome protected fragments (RPF) were purified by phenol-chloroform extraction, and suspended in Superase-IN AMBION solution (1 U/μL).

RPFs were purified on 16% (vol/vol) acrylamide-bisacrylamide (19:1) gel made with 7 M urea and 1× Tris-acetate-EDTA (TAE) buffer. After staining for 30 min in 1× SYBR Gold (Life Technologies) diluted in 1× TAE, RNA contained in the 28- and 34-nt regions were separately excised from the gel. The gels slices were stored at least 2 h at −20 °C and then physically disrupted. RNAs were eluted overnight from gel fragments by passive diffusion in 500 μL of RNA extraction buffer II (300 mM NaOAc pH 5.5, 1 mM EDTA) at 4 °C on a rotating wheel. After filtration, RPFs were precipitated in ethanol with 20 mg glycogen, suspended in 20 μL Superase-IN AMBION solution (1 U/μL), and stored at −80 °C until use.

Ribosome footprints were depleted from ribosomal RNA with the Ribo-Zero Human kit (Illumina) following the manufacturer's recommendations. Sequencing libraries were prepared from an equal number of RPF with the TruSeq Small RNA library preparation kit (Illumina). Next generation sequencing was performed using a HisEq. 2500 single read 75.

Bioinformatic analysis: From raw data to alignment files. Raw data were first trimmed to remove the 3′ adapter sequence with CutAdapt 1.9.1 configured with –e 0.12, -m 24, -M 35 (-M 51 for RNA-seq) and -a options to select a read size in a range from 24 to 35 nt allowing 12% of error. The trimmed reads were then mapped against the rRNA sequences of hg38 human genome with Bowtie 1.1.2 set up with –all and –un options. The –un option was used to select all unmapped reads. These filtered reads were finally mapped against the complete genome (hg38 human genome) and against the coding sequences only with Bowtie 1.1.2. The latter alignments were configured with two mismatches allowed (-n 2) and only uniquely mapped reads were selected (-m 1). The sam formatted files generated by the aligner were converted to sorted and indexed bam-formatted files using the Samtools program.

Differential expression. The number of reads for each gene was calculated with the featureCounts 1.5.0-p2 program and normalized with DESeq2 method through SARTools R Package. GO terms were identified for genes showing a significant expression variation using Panther (43). To represent RNA-seq and RIBO-seq profiles, we used the cross graphic representation where we display log_2 fold-changes in both data. The data were directly taken from the tables generated by SARTools. The colors were then selected based on the Panther groups and manually curated in case of no panther group was identified. The graphic was done using Bokeh v0.12.9 (https://bokeh.pydata.org/en/0.12.9/).

Ribosome footprints repartition. To identify reads as ribosome footprints, we did a metagene over the transcripts of hg38 human assembly to study the periodicity and the proportion of reads mapped on the CDS, UTR5, and UTR3. This analysis was done using only the 28 Mers, which are the most abundant kmers. Only transcripts coding for proteins with "appris_principal" tag were kept which represent 26,176 transcripts. For the metagene, we selected the 100 last nucleotides of the UTR5, the 100 first nucleotides of the UTR3 and the 100 first and 100 last nucleotides of the CDS. All transcripts that didn't have one of these three requirements was discarded for the metagene analysis. With these filters, we analyzed 25,062 CDS and 14,859 UTR5/3. From this metagene, we counted the proportion of mapped reads (28Mers) and realized a Fourier transform using the scipy package with the scipy.fft.fft function to study the periodicty. Python Bokeh library v0.12.9 was used to plot the percentages of mapped 28Mers on each feature and their corresponding period detected from Fourier transform results.

In vitro hybrid translation. Hybrid in vitro translation assay was performed as described previously (35) and is summarized hereafter. After centrifugation of 1 mL of RRL for 2 h 15 min at 240,000 × *g*, 900 μL of ribosome-free RRL (named S100) was collected, frozen, and stored at −80 °C. The extent of ribosome depletion from reticulocyte lysate was checked by translating 27 nM of in vitro transcribed capped and polyadenylated globin-*Renilla* mRNA in the S100 RRL and validated when no luciferase activity could be detected. In parallel, transfected cells were lysed in hypotonic buffer R [Hepes 10 mM pH 7.5, CH_3CO_2K 10 mM, $(CH_3CO_2)_2Mg$ 1 mM, DTT 1 mM] and potter homogenized (around 100 strokes). Cytoplasmic fraction was obtained by 13,000 × *g* centrifugation for 10 min at 4 °C. The ribosomal pellet was then obtained by ultracentrifugation for 2 h 15 min at 240,000 × *g* in a 1 M sucrose cushion and was rinsed three times in buffer R2 containing Hepes 20 mM, NaCl 10 mM, KCl 25 mM, $MgCl_2$ 1.1 mM, β-mercaptoethanol 7 mM and suspended in 30 μL of buffer R2 to reach more than 10 μg/μL ribosome concentration for optimal and long storage at −80 °C. The reconstituted lysate was then assembled by mixing 5 μL of S100 RRL with a scale from 0.25 to 4 μg of ribosomal pellet. Typically, the standard reaction contained 5 μL of ribosome-free RRL with 1 μg ribosomal pellet in a final volume of 10 μL. Upon reconstitution, the translation mixture was supplemented with 75 mM KCl, 0.75 mM $MgCl_2$, and 20 μM amino acid mix.

For in vitro translation assays, p0-*Renilla* vectors containing the β-globin, GAPDH 5′UTR, CrPV, DCV, or EMCV IRESs were described previously (38). mRNAs were obtained by in vitro transcription, using 1 μg of DNA templates linearized at the AflII sites, 20 U of T7 RNA polymerase (Promega), 40 U of RNAsin (promega), 1.6 mM of each ribonucleotide triphosphate, 3 mM DTT in transcription buffer containing 40 mM Tris·HCl (pH 7.9), 6 mM $MgCl_2$, 2 mM spermidine, and 10 mM NaCl. For capped mRNAs, the GTP concentration was reduced to 0.32 mM and 1.28 mM of m7GpppG cap analog (for β-globin mRNA) or m7GpppA (for CrPV mRNA) (New England Biolabs) was added. The transcription reaction was carried out at 37 °C for 2 h, the mixture was treated with DNase and the mRNAs were precipitated with ammonium acetate at a final concentration of 2.5 M. The mRNA pellet was then suspended in 30 μL of RNase-free water and mRNA concentration was determined by absorbance using the Nanodrop technology. mRNA integrity was checked by electrophoresis on nondenaturing agarose gel.

Statistical Analysis. Statistical analysis was performed using the Prism software (v7.0. GraphPad). A two-tailed Student *t* test was used for evaluating significance, except for RiboMethSeq data for which a one-tailed *t* test was used.

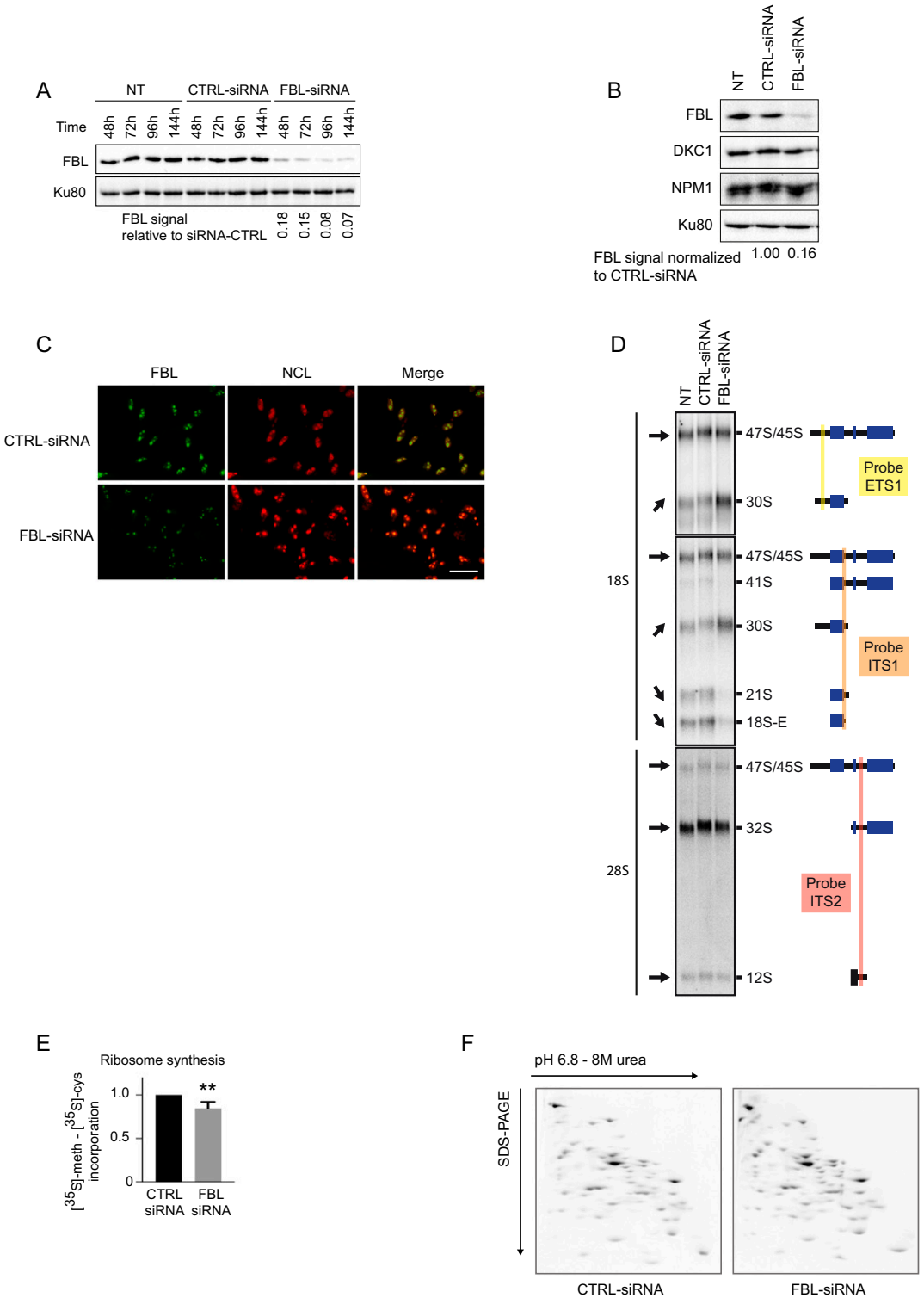

Fig. S1. Related to Fig. 1. Impact of FBL knockdown on major nucleolar markers. (*A*) Western blot analysis of *FBL* in HeLa cells transfected with three siRNAs targeting FBL for the periods of time indicated above each lane. Ku80 was used as a loading control. FBL signal was quantified and normalized against CTRL-siRNA (values are indicated below for each condition). (*B*) Western blot analysis of dyskerin (DKC1) and nucleophosmin (NMP1) levels in FBL knockdown HeLa cells compared with nontransfected cells (NT) and cells transfected with a CTRL-siRNA. Analysis performed 72 h posttransfection. Ku80 was used as a loading control. FBL signal was quantified and normalized against the CTRL-siRNA condition. (*C*) Immunofluorescence detection of FBL (green) and nucleolin (NCL) (red) 72 h after transfection with CTRL or FBL-siRNA. (Scale bar, 10 μm.) (*D*) Northern blot analysis of pre-rRNA processing in nontransfected (NT) or siRNA transfected (CTRL-siRNA and FBL-siRNA) HeLa cells. The position of the probes used and the detected prerRNA species are indicated on the right. Arrows on the left indicate the trend of each species in FBL-siRNA cells to increase or decrease compared with CTRL-siRNA cells. (*E*) Ribosome synthesis rate. [^{35}S]-Meth–[^{35}S]-Cys incorporation into purified cytoplasmic ribosomes 72 h after siRNA transfection in HeLa cells. Radioactivity was measured by liquid scintillation. Values are presented as mean ± SD ($n = 2$). **$P \leq 0.01$. (*F*) The 2D-PAGE analysis of 0.5 M KCl-purified ribosomes extracted from [^{35}S]-Meth–[^{35}S]-Cys pulse-labeled cells. Proteins were separated according to their charge in the first dimension and according to their molecular weight in the second dimension. Images show the radioactive signal obtained by phosphor-imaging.

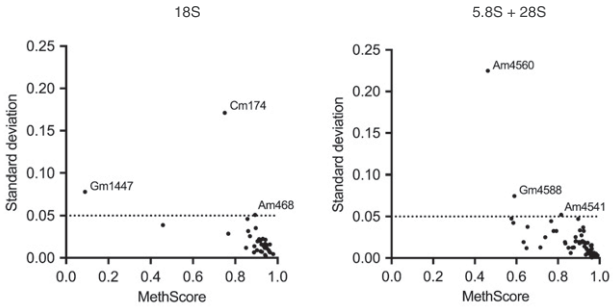

Fig. S2. Related to Fig. 2. Quantitative mapping of rRNA 2′-O-Me in human cells. Variability (SD) of RiboMethSeq data for each 2′-O-Me site, according to its level of methylation. Sites with variability greater than 5% are named on the graph. Most sites show a variability below 5% (dotted line). Values are represented as means of three independent biological replicates.

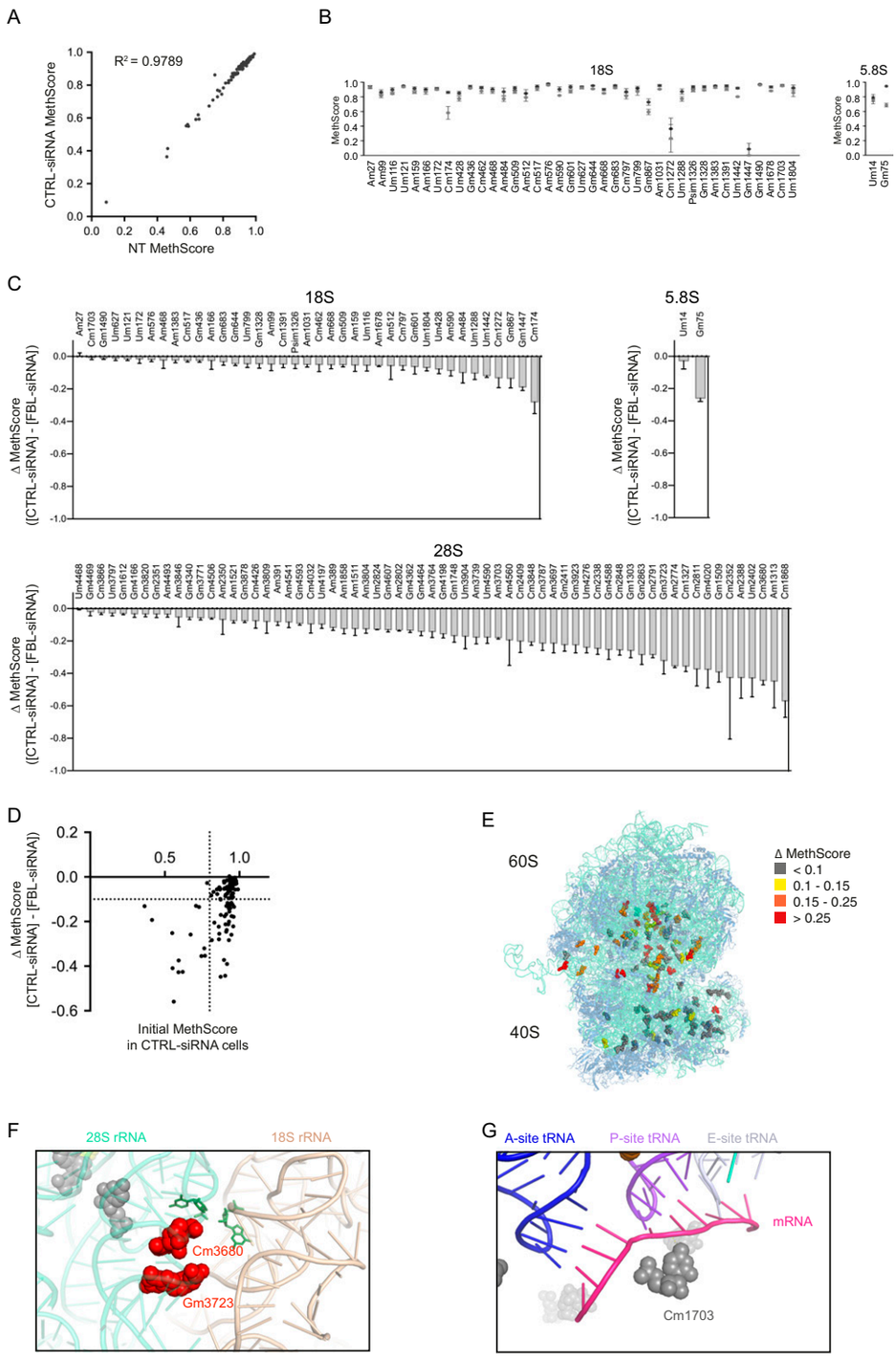

Fig. S3. Related to Fig. 3. Identification and localization of altered 2′-O-Me sites. (*A*) siRNA transfection has no effect on 2′-O-Me. Plot of the MethScore of each site in nontreated HeLa cells (NT) vs. HeLa cells transfected with control siRNA (CTRL-siRNA). Correlation coefficient (R2) was calculated from the linear regression curve. (*B*) Alteration of 2′-O-Me upon *FBL* knockdown. Mean MethScore values ± SD ($n = 3$ independent biological replicates) for each methylated nucleotide in 18S and 5.8S rRNA from HeLa cells transfected with CTRL-siRNA (black circle) or FBL-siRNA (gray circle). (*C*) FBL knockdown induces a decrease of MethScore at all but one site. For each site, the MethScore obtained in FBL-siRNA transfected HeLa cells was subtracted from the one from CTRL-siRNA transfected cells (same dataset as in Fig. 2*B*). Sites are shown in order of increasing difference in MethScore for the 18S, 5.8S, and 28S rRNAs. Error bars are SD from three independent biological replicates. (*D*) Distribution of 2′-O-Me sites according to their initial methylation frequency (*x* axis) and to their methylation variation upon FBL knockdown (*y* axis). Partially methylated sites in siRNA-CTRL cells (left of the vertical dotted line) were all down-methylated in siFBL-cells (ΔMethScore > 0.1). Note that the site 18S-Cm1447 was excluded, since it is not methylated our HeLa cells. (*E*) Position of methylated nucleotides on the 3D structure of the human ribosome, shown as assembled 80S ribosome. Nucleotides are color-coded according to the variation in MethScore comparing FBL-siRNA cells with CTRL-siRNA cells. See also Datasets S2 and S3. (*F*) View of intersubunit bridge B2b [18S-1051 and 28S-3699 (shown as stick in green)], showing close proximity of Cm3680 and Gm3723 methylation sites (red), which methylation was decreased by 44.3% and 32.0%, respectively. (*G*) View of the decoding center, showing the anticodon loop of tRNAs interacting with the mRNA at the A, P, and E-sites. The Cm1703 nucleotide (gray, not affected by FBL knockdown) is close to the mRNA.

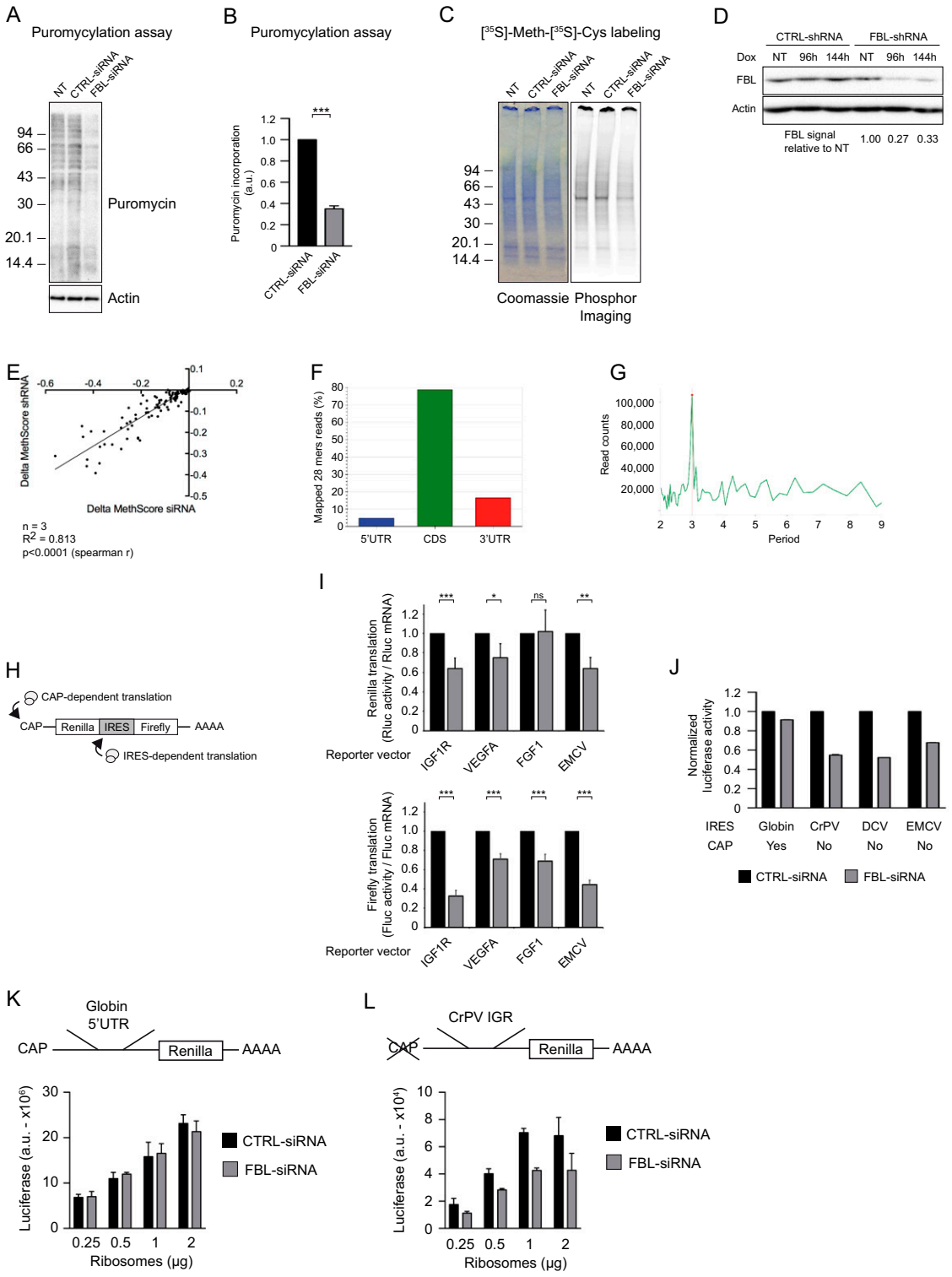

Fig. S4. Related to Fig. 4. rRNA methylation defect impairs IRES dependent translation from various viral IRES elements. (*A*) Global protein synthesis detection by incorporation of puromycin in nascent peptides. A representative Western blot with an antipuromycin antibody is shown. Actin detection was used as loading reference. (*B*) Global protein synthesis was measured by incorporation of puromycin in nascent peptides using Western blot against puromycin. Puromycin signal was normalized against actin. Values are presented as mean ± SD ($n = 3$). (*C*) Global protein synthesis detection by pulse labeling with [^{35}S]-methionine and [^{35}S]cysteine. A representative SDS/PAGE stained with Coomassie is shown (*Left*). The corresponding radioactive signal is shown upon phosphor-Imaging detection (*Right*). (*D*) Western blot analysis of *FBL* level in HeLa cells in which the expression of a CTRL shRNA or a FBL-shRNA was induced for 120 h by doxycycline (Dox) treatment. Actin was used as a loading control. The level of FBL upon shRNA expression is indicated below and normalized to CTRL-shRNA. (*E*) Plotting of the decrease of 2′-O-Me (ΔMethScore) in FBL-shRNA expressing cells (*y* axis) and of the ΔMethScore in FBL siRNA transfected cells (*x* axis). The data shows that the change in pattern of 2′-O-Me is similar in shRNA expressing cells compared with siRNA expression cells (Spearman *r* test, $P <$ 0.0001). (*F*) Fraction of the mapped 28Mers on each feature of transcripts. In this metagene, transcripts with a CDS length lower than 200 nucleotides and a 5′

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UTR and 3′UTR lengths lower than 100 nt were discarded. (*G*) Period detected from the frequencies of RPF obtained by fast Fourier transform. The red dot shows the most frequent period found in the data. The vertical red line shows the expected period of 3 nt for ribosome signals. This periodicity is not observed in UTRs regions indicating that the RPF correspond to active ribosome footprints. (*H*) Representation of in cellulo IRES-dependent translation assay using a bicistronic luciferase reporter construct. *Renilla* luciferase is translated in a Cap-dependent manner and Firefly luciferase is translated from the IRES element. (*I*) Translation efficiency of individual luciferase reporters was evaluated as the ratio of luciferase activity over mRNA levels. Luciferase activities were measured as in Fig. 4 and compared with luciferase mRNA levels measured by RT-qPCR. Data represent activity/mRNA ratios for Rluc (*Upper*, gray bars) and Fluc (*Lower*, gray bars), normalized against CTRL-siRNA (black bars). Values are presented as mean ± SD ($n = 3$). (*J*) Ribosomes purified from HeLa cells transfected with either CTRL-siRNA or FBL-siRNA were used to translate mRNAs in the hybrid in vitro translation assay. Translation was evaluated on luciferase reporter mRNAs containing a 5′UTR originating from human globin mRNA, or containing IRES elements from the CrPV, the DCV, or EMCV. The presence of Cap on the mRNA is indicated below the graph. Data are presented as mean ± SD ($n = 2$). (*K* and *L*) Identical experimental set-up as in Fig. 4*F* using a range of ribosome quantities. Data represent mean values (±SD, $n = 3$). Statistical significance was verified by conducting a Student *t* test. ns, not significant; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)