Supporting Information

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SI Methods

Oocytes. Immature, folliculated stage I–VI, defolliculated, and matured oocytes were manipulated as described previously (1). Nuclei were injected with 18 nL of reporter, RNA, or plasmid using the Drummond Nanojet II system. Initially 37.5 ng of control (CCCAGGGACCTCTCTCTAATCA) or eIF5B antisense (CACTCTTGGTCTTCTGTTTCTTCCC), or eIF2γ (CCCGGCTCGTCGCCCGCCATCTT) antisense along with 0.125 ng of pcDNA3, GFP (vector control), cytomegalovirus (CMV) plasmids expressing human eIF5B or eIF4G3, or 5–10 ng of their transcribed RNAs per oocyte were injected at the nuclear pole followed by a 6- to 7-h incubation. At these concentrations eIF5B was depleted in stage IV and V but not in stage VI oocytes, likely because of the increased stability of the protein; therefore, stage VI oocytes were not used for analysis. After incubation for 6 h, 18 nL of Firefly luciferase containing 0.125 ng plasmid reporter DNA was injected into the oocyte nucleus. Translation was assayed after 4 h and was conducted primarily with folliculated stage V oocytes (1).

All RNA and protein extracts were prepared after collagenase treatment followed by manual defolliculation and examination to ensure the complete defolliculation of oocytes (1, 2) to prevent contamination of the samples with follicular material. For all gels, 20 oocytes, isolated as described (1, 2), were run in each lane.

Plasmids and Transfections. pTRIPZ (Open Biosystems, Thermo) constructs against eIF5B, both a human-specific shRNA (V2THS_134249) and a second human- and mouse-specific shRNA (V2THS_134253), eIF2α, eIF2β, eIF2γ, and CMV-SPORT6 plasmids encoding eIF5B and eIF4G3 were obtained from Open Biosystems. All other plasmids and reporters used were described previously (3). Transient transfections were conducted using Nucleofector (Lonza) in THP1 cells and Lipofectamine 2000 (Invitrogen) in stem cells. The pTRIPZ vector, which expresses an shRNA with miR30a primiR sequences, was used as control and was transiently transfected in parallel with eIF5B and eIF2γ shRNAs. Stable cell lines were constructed as described by Open Biosystems. For eIF5B knockdown in THP1, the two stable shRNA eIF5B cell lines either were not induced (control) or were induced with 1 μg/mL doxycycline in serum-containing medium for 3 d before transfer to serum-free medium for 1 d. Cell growth was analyzed by averaging triplicate cell counts of live cells after trypan blue staining.

Luciferase Assay. Extract preparation and luciferase assays were performed as described in the manufacturer's protocol (Promega). The average ratios of Luciferase values from at least three biological replicate experiments are depicted with the SDs or SEM as error bars. The luciferase activity was normalized to total nucleic acid values, representing relative cell numbers, or to total oocyte numbers, to ensure that equal numbers of cells or oocytes were compared.

MTS Assay. The MTS assay, a colorimetric assay for cell growth, measuring cellular NAD(P)H-dependent oxidoreductases that reduce a tetrazolium dye, MTS, in the presence of phenazine methosulfate, was conducted as described by the manufacturer, Promega.

Quantitative RT-PCR Analyses. cDNA synthesis was performed using Random Primers (Invitrogen), and the cDNA was subjected to PCR amplification with annealing at 52 °C and 25–35 cycles for various cDNA preparation yields. Quantitative real-time PCR (qRT-PCR) was performed following the manufacturer's directions (Invitrogen). For mammalian samples, primers to endogenous methionyl tRNA (tRNA-Met_i), (tRNA-Met_i-5: GCAGAGTGGCGCAGCGGAAGCGTG, and tRNA-Met_i-3: CGAAACCATCCTCTGCTA) were used. As a loading control, primers to tRNA-Lys (tRNA-Lys-5: GCCCGGATAGCTCAGTCGGTAGAG and tRNA-Lys-3: CGCCCGAACAGGGACTTGAACCC) were used. For exogenously introduced Firefly, FF-F3 (TTCCATCTTCCAGGGATACG) and FF-R3 (ATCCAGATCCACAACCTTCG) were used. Renilla (Ren1: (CCATGATAATGTTGGACGAC; Ren2: GGCACCTTCAACAATAGCATTG) was used as an alternative control.

Cross-Linking, Immunoprecipitation, and Western Blot Analyses. Nuclear–cytoplasmic fractionation was performed as described previously (3), and the two fractions were combined for extracts for Western blot analysis and formaldehyde cross-linked immunoprecipitation samples. The samples were precleared with anti-myc agarose and were clarified by centrifugation during which very large cross-linked complexes were centrifuged down and excluded. Anti-eIF5B (Bethyl Antibodies) was used for immunoprecipitation. eIF5B is known to be phosphorylated (4) and runs as multiple bands, representing the dephosphorylated form in the extracts to which phosphatase inhibitors were not added. eIF5B is also known to form a degradation product (Fig. S3*A*) (5, 6). Phospho-Cdc2 runs variably as multiple bands due to modifications that include multiple-site phosphorylated forms in the extracts to which phosphatase inhibitors were not added [Cdc2 is phosphorylated at multiple sites (7)] and due to a variably observed truncation that migrates as a lower band and was observed previously (figure S5 in ref. 1). The lack of phosphatase inhibitors in the extracts and the set amount of Cdc2 in these oocytes would limit further increase in phospho-Cdc2 levels upon eIF5B depletion. All bands were quantitated. All replicates show the same trend, and the average of multiple experiments are shown. Blots were probed with anti-eIF5B and anti-eIF2γ (Protein Tech), GFP (Roche), anti-ribosomal protein S3 (RPS3), anti-Tubulin, anti–phospho-eIF2α, anti-eIF2Bγ, and anti-eIF2β (Santa Cruz), anti-actin (Sigma), and anti-eIF2α, anti-Oct4, anti-Nanog, anti-Sox2, and anti–phospho-CDC2 (Cell Signaling).

^{35}S-Methionine Incorporation Analysis. The indicated THP1 stable cell lines were grown in normal RPMI medium to prevent additional cellular stress from methionine-free medium. Then 100 μCi of ^{35}S-methionine was added to 10 mL of THP1 cells. After incubation at 37 °C for 45 min, cells were washed once with PBS and were resolved in 500 μL of SDS loading buffer. Radioactive SDS/PAGE gels were exposed to the phosphoimager (GE Healthcare) and quantified by ImageJ.

Polysome Profiling and Microarray. Sucrose solutions [15% and 50% (wt/vol)] were prepared in buffer A [10 mM Tris·HCl (pH 7.4), 100 mM KCl, 5 mM $MgCl_2$, 100 μg/mL cycloheximide, and 2 mM DTT]. Sucrose density gradients were prepared as previously described (8). Before harvesting, eIF5B-depleted cells and nondepleted control cells grown in serum-containing medium or grown for 1 d under serum starvation were treated with 100 μg/mL cycloheximide at 37 °C for 5 min. Collected cells were washed with cold PBS containing cycloheximide and then were lysed in buffer A containing 1% Triton X-100 and 40 U/μL murine RNase Inhibitor (New England Biolabs). Cleared cell lysates were loaded on sucrose gradients followed by ultracentrifugation

(Beckman Coulter Optima L90) for 2 h at 182,348 × *g* at 4 °C in an SW40 rotor. Samples were fractionated by a density gradient fractionation system (Isco). Total RNA was extracted with three volumes of TRIzol (Invitrogen) from the heavy polysome fractions [three or more polysomes (9, 10)] that were pooled. The synthesized cDNA probe (WT Expression Kit; Ambion) was hybridized to Gene Chip Human Transcriptome Array 2.0 (Affymetrix) by the Partners Healthcare Center for Personalized Genetic Medicine Microarray facility. A 1.5-fold change in microarray expression was used as the cutoff to determine differentially regulated genes. Gene Ontology (GO) analysis for differentially expressed genes was performed with the DAVID tool (11). Microarray results related to Figs. 3 and 4, Fig. S7, and Table S2 are provided in Dataset S1.

1. Mortensen RD, Serra M, Steitz JA, Vasudevan S (2011) Posttranscriptional activation of gene expression in Xenopus laevis oocytes by microRNA-protein complexes (microRNPs). *Proc Natl Acad Sci USA* 108(20):8281–8286.
2. Moon KH, Zhao X, Yu YT (2006) Pre-mRNA splicing in the nuclei of Xenopus oocytes. *Methods Mol Biol* 322:149–163.
3. Truesdell SS, et al. (2012) MicroRNA-mediated mRNA translation activation in quiescent cells and oocytes involves recruitment of a nuclear microRNP. *Sci Rep* 2:842.
4. Beausoleil SA, et al. (2004) Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc Natl Acad Sci USA* 101(33):12130–12135.
5. White JP, Reineke LC, Lloyd RE (2011) Poliovirus switches to an eIF2-independent mode of translation during infection. *J Virol* 85(17):8884–8893.
6. de Breyne S, Bonderoff JM, Chumakov KM, Lloyd RE, Hellen CU (2008) Cleavage of eukaryotic initiation factor eIF5B by enterovirus 3C proteases. *Virology* 378(1):118–122.
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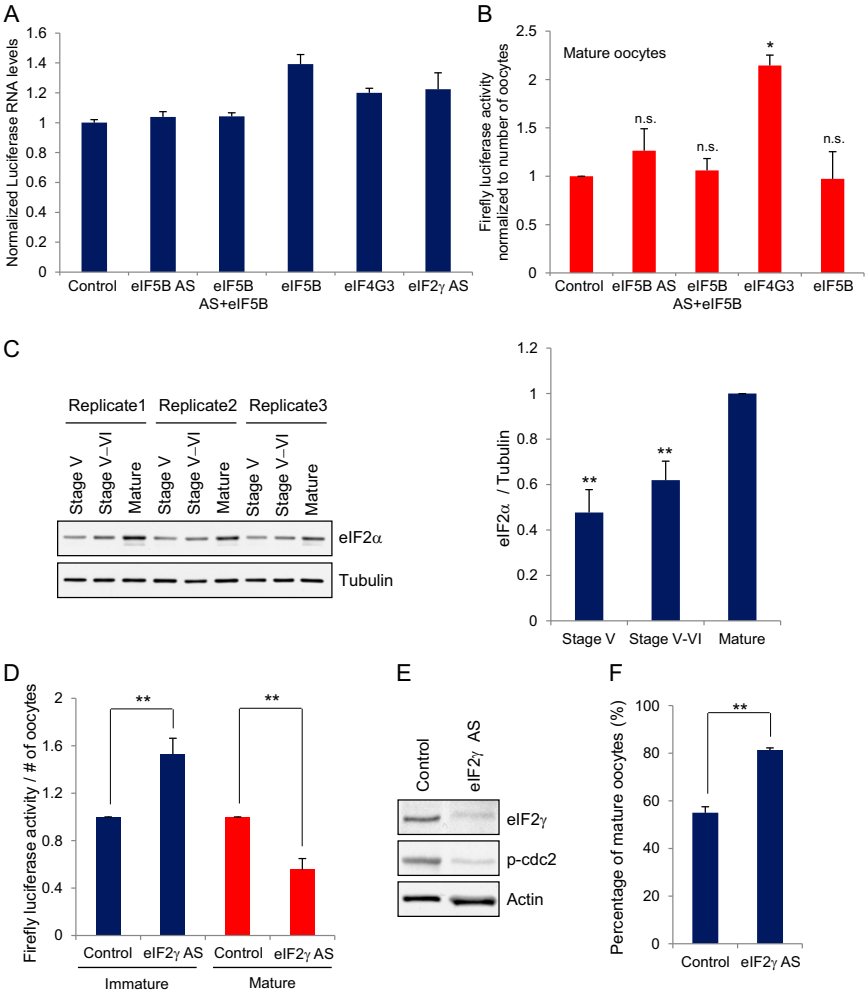

Fig. S1. (Related to Fig. 1) eIF5B depletion does not affect translation in mature oocytes, and eIF2 is not limiting for translation in late immature oocytes. (*A*) qRT-PCR of luciferase mRNA levels normalized to tRNA-Lys in oocytes injected with GFP vector control+control antisense (Control), vector+eIF5B antisense (eIF5B AS), eIF5B mRNA+eIF5B antisense (eIF5B AS+eIF5B), control antisense+eIF5B mRNA (eIF5B), control antisense+eIF4G3 mRNA (eIF4G3), or vector control+eIF2γ antisense (eIF2γ AS). After 6 h, these oocytes were injected with Firefly luciferase reporter plasmid to measure the effects of eIF5B depletion, rescue, or overexpression or that of the control translation factor, eIF4G3, or of eIF2γ antisense on RNA levels. Similar results were seen when oocytes that matured were separated. No significant differences in RNA levels were observed. (*B*) Oocytes were injected with vector+control antisense (Control), vector+eIF5B antisense (eIF5B AS), eIF5B mRNA+eIF5B antisense (eIF5B AS+eIF5B), eIF4G3 mRNA+control antisense (eIF4G3), or eIF5B mRNA+control antisense (eIF5B). After 6 h, Firefly luciferase reporter plasmid was injected in these oocytes, and mature oocytes were isolated to measure the effects of eIF5B depletion, rescue, or overexpression or that of the control translation factor, eIF4G3, on general translation in mature oocytes. (*C*) Total eIF2α levels shown by Western blot analysis in a comparison of 20 oocytes from immature stage V and V–VI and mature oocytes (*Left*) and their quantitation (*Right*). These lysates were collected from the bottom of the tube with a 22-G, 1.5-inch needle to get rid of lipids that led to the nonspecific band observed with eIF2α antibody in Fig. 1*A*. Tubulin was used as a loading control. (*D*) Immature oocytes were injected with vector+control antisense (Control) or with vector+eIF2γ antisense (eIF2γ AS) and then were injected with a Firefly luciferase reporter plasmid to measure the effects of eIF2γ levels on general translation in stage V immature oocytes and matured oocytes. (*E*) Western blot of eIF2γ depletion and the marker for immaturity, phospho-Cdc2 (p-cdc2) (1). Actin was used as a loading control. (*F*) Immature oocytes injected with vector+control antisense (Control) or with vector+eIF2γ antisense (eIF2γ AS) were scored for germinal vesicle breakdown to measure the effects of eIF2γ levels on maturation (Table S1) (1). The graph shows the average of three experiments; error bars indicate SEM. n.s., not significant. $*P < 0.05$; $**P < 0.01$.

1. Mortensen RD, Serra M, Steitz JA, Vasudevan S (2011) Posttranscriptional activation of gene expression in Xenopus laevis oocytes by microRNA-protein complexes (microRNPs). *Proc Natl Acad Sci USA* 108(20):8281–8286.

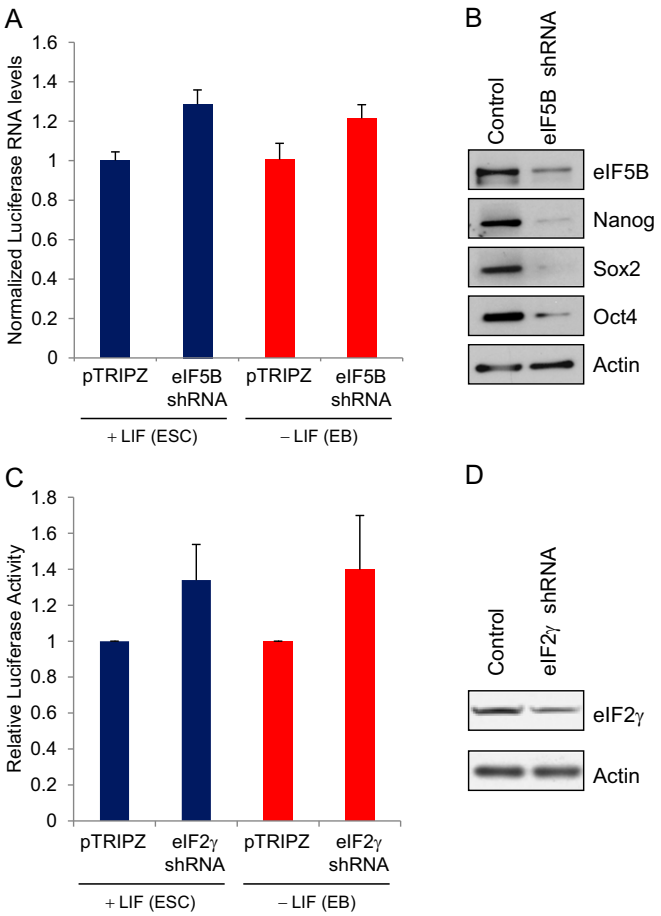

Fig. S2. (Related to Fig. 2) Effect of eIF5B and EIF2γ depletion on translation in stem cells. (*A*) RNA levels of samples from Fig. 2*C* are shown. qRT-PCR of luciferase mRNA levels normalized to tRNA-Lys in ES cells and embryoid bodies (EBs) without (pTRIPZ; control shRNA) and with eIF5B depletion (eIF5B shRNA). No significant differences in RNA levels were observed between control and eIF5B knockdown cells. Cells were transfected with luciferase reporter plasmid and either control pTRIPZ or eIF5B shRNA vectors. Knockdown was induced in the transfected cells with doxycycline administered for 3 d in ES cells (+ LIF) or for 3 d after differentiation into EBs (11 d −LIF). (*B*) Western blot of Nanog, Sox2, Oct4, and eIF5B in ES cells transfected with pTRIPZ shRNA (Control) or eIF5B shRNA vectors and induced with doxycycline for 3 d to express the shRNAs in ES cells. Actin was used as a loading control. (*C*) Luciferase reporter assay normalized to nucleic acid levels of ES cells (+LIF) and EBs (11 d −LIF), transfected with luciferase reporter plasmid and either pTRIPZ (Control) or eIF2γ shRNA. Error bars indicate SEMs from three independent experiments. No significant differences were observed. (*D*) Western blot of stem cells transfected with pTRIPZ (Control) shRNA vector or eIF2γ shRNA and induced with doxycycline to express the shRNAs as described for eIF5B knockdown in *A* and *B*. Pooled ES cells and EBs were checked for depletion. Actin was used as a loading control. LIF, leukemia inhibitory factor.

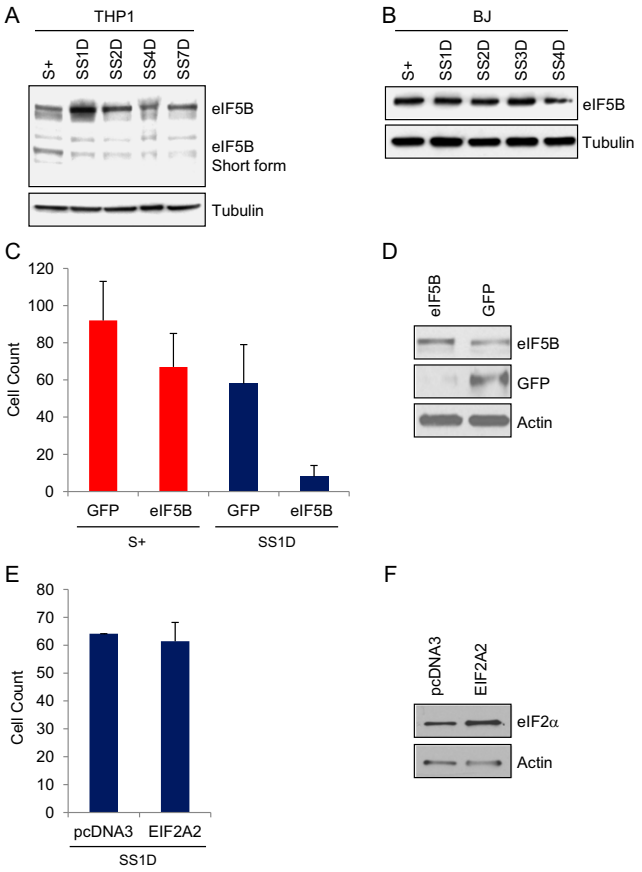

Fig. S3. (Related to Fig. 3) eIF5B levels are increased transiently in THP1 cells that had been serum-starved for 1 d in which additional overexpression of eIF5B promotes cell death. (*A*) Western analysis of eIF5B levels over days of serum starvation (SS1D, SS2D, SS4D, and SS7D) compared with serum-grown (S+) cells from Fig. 3*A* also depicting the lower truncated product (1, 2) observed mostly in serum-grown cells. eIF5B is known to be phosphorylated (3) and runs variably as multiple bands, representing the dephosphorylated form in these extracts to which phosphatase inhibitors were not added. (*B*) eIF5B levels do not change in BJ cells upon serum starvation over time. Tubulin was used as a loading control. Other eIF2α-alternative factors, eIF2A and eIF2D (4–6), were tested but did not show any increase upon serum starvation. (*C*) Overexpression of eIF5B and GFP in serum-grown and serum-starved cells. Cell counts were plotted relative to day 0 cell counts to measure cell proliferation; the serum-starved eIF5B-overexpressing cells show rapid cell death within 1 d of serum starvation. (*D*) Western blots showing the overexpression of eIF5B (1.5 ± 0.14) and GFP expression in *C*. Actin served as a loading control. (*E*) Overexpression of eIF2α (the eIF2αS51A nonphosphorylatable form, called "eIF2A2," is shown) and the vector control pcDNA3 in serum-starved cells. Cell counts were plotted relative to day 0 cell counts to measure cell proliferation; the serum-starved eIF2α-expressing cells are viable, and no significant differences in cell counts between control and eIF2α-overexpressing cells were seen over 1 d of serum starvation of THP1 cells. (*F*) Western blots showing the overexpression of eIF2α (2.4 ± 0.18) in *E*. Actin served as a loading control. Graphs in *C* and *E* show the average of three experiments; error bars show SEM.

1. White JP, Reineke LC, Lloyd RE (2011) Poliovirus switches to an eIF2-independent mode of translation during infection. *J Virol* 85(17):8884–8893.
2. de Breyne S, Bonderoff JM, Chumakov KM, Lloyd RE, Hellen CU (2008) Cleavage of eukaryotic initiation factor eIF5B by enterovirus 3C proteases. *Virology* 378(1):118–122.
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5. Kim JH, Park SM, Park JH, Keum SJ, Jang SK (2011) eIF2A mediates translation of hepatitis C viral mRNA under stress conditions. *EMBO J* 30(12):2454–2464.
6. Dmitriev SE, et al. (2010) GTP-independent tRNA delivery to the ribosomal P-site by a novel eukaryotic translation factor. *J Biol Chem* 285(35):26779–26787.

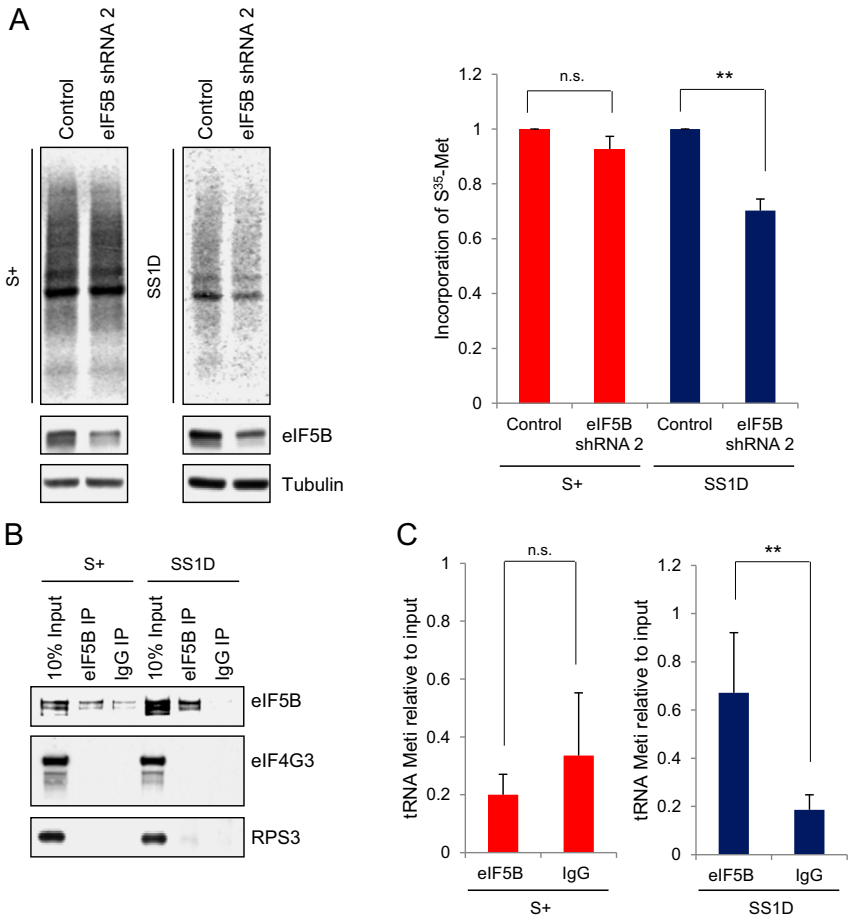

Fig. S4. (Related to Figs. 3 and 4) eIF5B depletion decreases translation in serum-starved cells in which the increased eIF5B levels lead to correspondingly increased complexes with tRNA-Met$_i$. (*A*) Incorporation of ^{35}S-methionine in THP1 cells without (Control) and with eIF5B depletion using a second eIF5B shRNA (eIF5B shRNA2), in serum-grown cells (S+) and in cells that were serum-starved for 1 d (SS1D) as in Fig. 4*A*. (*Upper Left*) Equal amounts of extracts, separated by SDS/PAGE, were exposed on the phosphorimager. (*Lower Left*) Western blots depict the level of eIF5B depletion. The ^{35}S-methionine incorporation in each sample was normalized to Tubulin (a stable, abundant protein) for loading. (*Right*) ^{35}S-methionine incorporation was quantified. The graph represents the average of three experiments. Error bars indicate SEM. n.s., not significant. (*B* and *C*) eIF5B and, as a control, IgG were immunoprecipitated from in vivo cross-linked cells from serum-grown (S+) THP1 cells and from THP1 cells that had been serum-starved for 1 d (SS1D), and the extracts were prepared as described in *SI Methods*, followed by qRT-PCR analysis for tRNA-Met$_i$. Values for tRNA-Lys were below significance. (*B*) Western blot of eIF5B immunoprecipitation reprobed to check for eIF4G3 and RPS3 coimmunoprecipitation. The difference between immunoprecipitated eIF5B protein levels, normalized to the input, is 0.33 (S+) and 0.42 (SS1D) for the immunoprecipitation replicate shown, and the average from three replicates is 0.32 (S+) and 0.49 (SS1D) with $P < 0.05$. This represents a 1.5-fold increase in eIF5B immunoprecipitated from serum-starved as compared with serum-grown cells. The variable background levels observed in control IgG immunoprecipitates from S+ and SS1D samples from three replicates average out to similar low values of 0.08 (S+) and 0.088 (SS1D) relative to the input. (*C*) Quantitation of tRNA-Met$_i$ coimmunoprecipitated with eIF5B and IgG control immunoprecipitation relative to input amounts from serum-grown (*Left*) and serum-starved (*Right*) cells. The increase in tRNA-Met$_i$ immunoprecipitation in serum-starved cells reflects increased eIF5B levels and increased eIF5B immunoprecipitated from serum-starved cells observed in *B*. The graph shows the average of three independent experiments. Error bars indicate SEM.

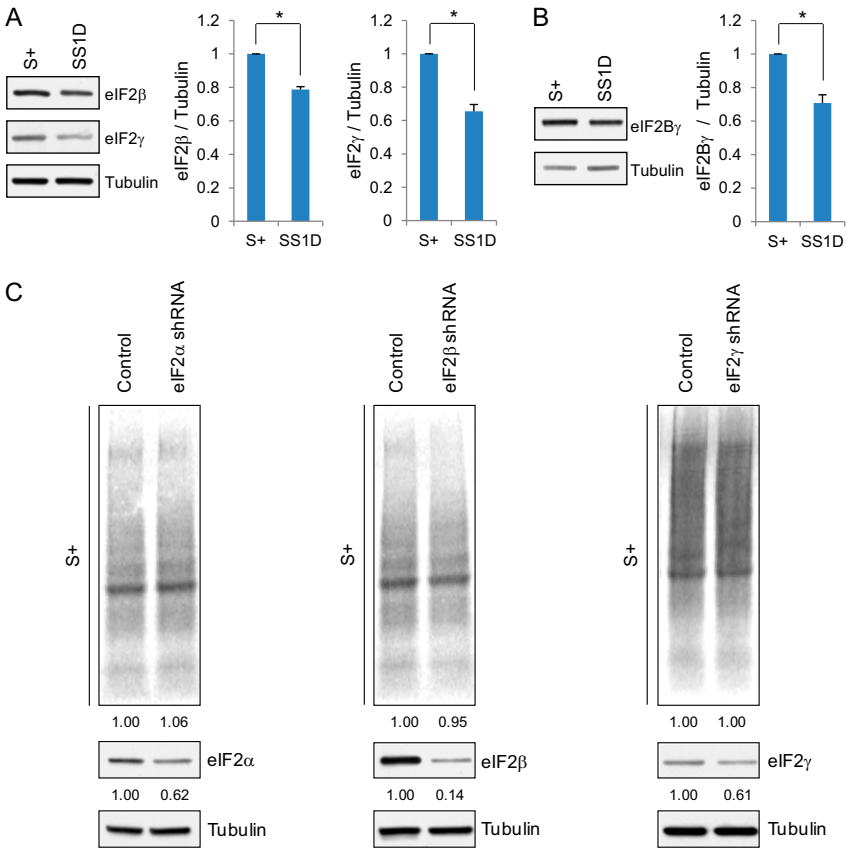

Fig. S5. (Related to Figs. 3 and 4) EIF2β, eIF2γ, and eIF2Bγ levels upon serum starvation and global translation analysis in eIF2α-, eIF2β-, and eIF2γ-depleted THP1 cells. (*A*, *Left*) Western blot analysis of eIF2β and eIF2γ levels in THP1 cells upon 1 d serum starvation (SS1D) compared with serum-grown cells (S+). (*Right*) Quantitation of eIF2β and eIF2γ levels from three experiments. (*B*) Western blot analysis of eIF2B (eIF2Bγ) levels in THP1 cells upon 1 d serum starvation compared with serum-grown cells (*Left*) and its quantitation (*Right*). (*C*) ^{35}S-methionine incorporation in THP1 cells without (Control) and with eIF2α, eIF2β, and eIF2γ knockdown, as described in *SI Methods*. Serum-grown cells (S+) are shown. Equal amounts of extracts, separated by SDS/PAGE, were exposed on the phosphorimager. The ^{35}S-methionine incorporation in each sample was normalized to Western analysis of Tubulin as a loading control. Western blot analysis depicting the level of eIF2 depletion, and their quantitation is shown. Graphs in *A* and *B* show the average of three independent experiments. Error bars indicate SEM. *$P < 0.05$.

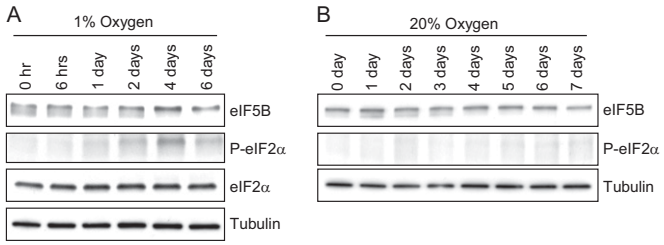

Fig. S6. (Related to Fig. 3) EIF2α phosphorylation and eIF5B levels in THP1 cells under hypoxia and normoxia. The levels of eIF2α phosphorylation and eIF5B are increased transiently at 4 d of hypoxia (*A*) but did not change significantly in normoxic conditions (*B*). Tubulin and total eIF2α were used as loading controls.

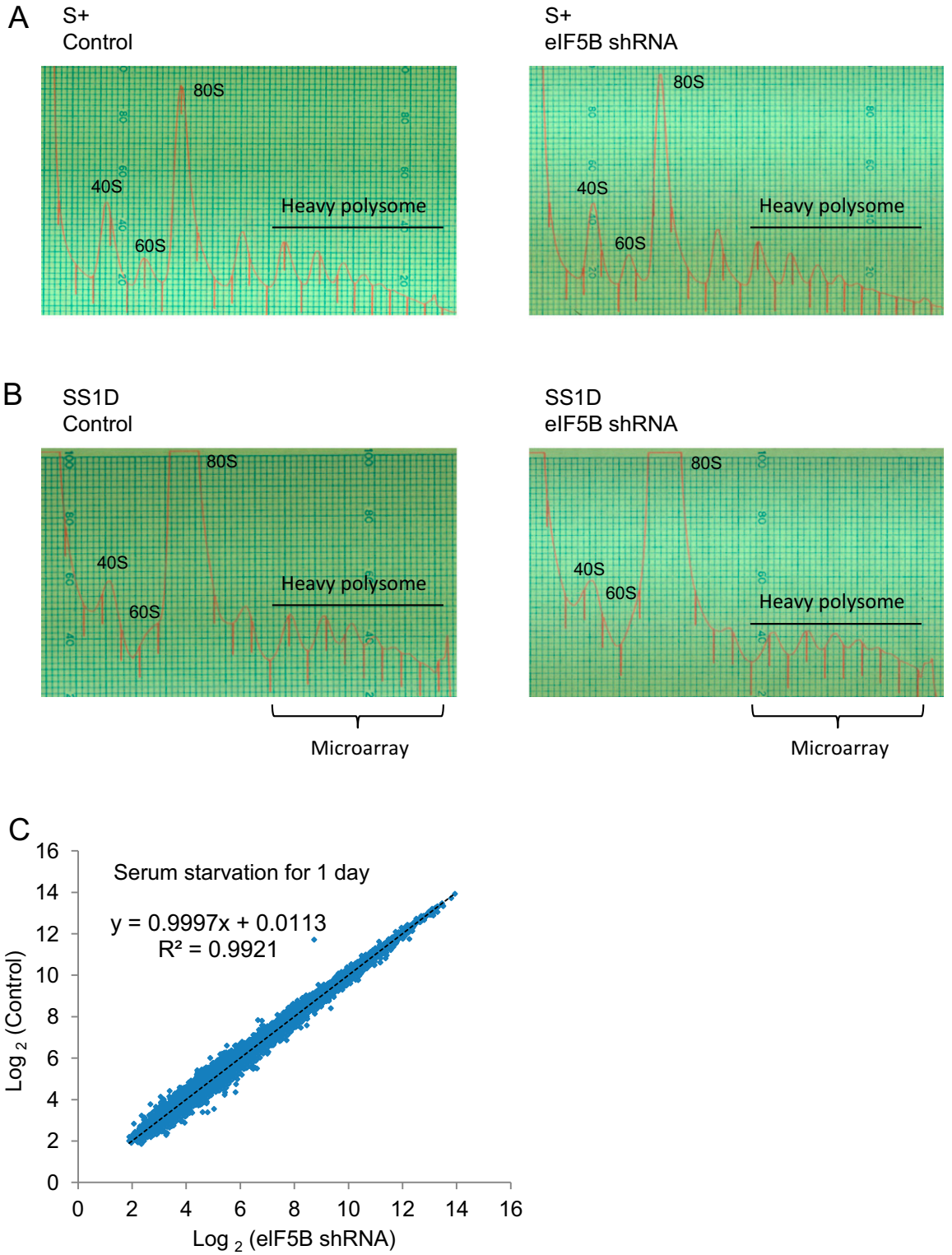

Fig. S7. (Related to Figs. 3 and 4) Genome-wide analysis of eIF5B-mediated translational regulation by comparison of eIF5B-depleted and control (undepleted) cells. (*A* and *B*) Polysome analysis. Polysome-associated RNAs were purified from undepleted control cells (Control) and eIF5B knockdown THP1 cells (eIF5B shRNA) from serum-grown (S+) (*A*) and cells that had been serum-starved for 1 d (SS1D) (*B*), followed by microarray analysis. (Microarray data are given in Dataset S1.) (*C*) The logarithmic value of individual RNAs in eIF5B knockdown cells was plotted against those values in undepleted cells.

Table S1. Effect of depletion, rescue, and overexpression of eIF5B on maturation of oocytes

	Control antisense + vector	eIF5B antisense + vector	eIF5B + eIF5B antisense	Control antisense + eIF5B	Control antisense + eIF4G3	eIF2γ antisense + vector
Rep I						
Mature	13	10	19	27	15	21
Immature	13	16	4	0	9	5
% mature	50	38	83	100	63	81
Rep II						
Mature	11	12	21	27	13	24
Immature	8	17	5	5	13	5
% mature	58	41	81	84	50	83
Rep II						
Mature	16	9	18	17	10	20
Immature	12	20	8	8	11	5
% mature	57	31	69	68	48	80

The number of immature and mature oocytes and percentage of mature oocytes observed in three biological replicate experiments with immature oocytes injected with: control antisense + vector (labeled control, GFP), eIF5B antisense + vector (labeled eIF5B antisense), eIF5B antisense + eIF5B, control antisense + eIF5B (labeled eIF5B), control antisense + eIF4G3 (labeled eIF4G3), and eIF2γ antisense + vector (labeled eIF2γ antisense) are shown. See Fig. 1 *D* and *G* and Fig. S1*F* for the percentage of immature oocytes. Rep, replicate.

Table S2. GO analysis of genes down-regulated upon eIF5B knockdown in THP1 cells that were serum-starved for 1 d

Type	Gene ontology category description	Number of genes	Benjamini *P* value
Cluster 1 (enrichment score: 1.75)			
BP	Sensory perception of chemical stimulus	7	5.7E-02
MF	Olfactory receptor activity	6	8.8E-02
BP	Sensory perception of smell	6	1.4E-01
BP	Sensory perception	7	2.6E-01
BP	Cognition	7	3.3E-01
BP	G protein coupled receptor protein signaling pathway	7	5.8E-01
BP	Neurological system process	7	5.8E-01
BP	Cell surface receptor linked signal transduction	8	7.9E-01
CC	Integral to membrane	11	1.0E+00
CC	Plasma membrane	8	1.0E+00
CC	Intrinsic to membrane	11	1.0E+00
Cluster 2 (enrichment score: 1.4)			
BP	Spermatogenesis	4	5.7E-01
BP	Male gamete generation	4	5.7E-01
BP	Gamete generation	4	7.0E-01
BP	Sexual reproduction	4	7.6E-01
BP	Multicellular organism reproduction	4	7.8E-01
BP	Reproductive process in a multicellular organism	4	7.8E-01

Microarray analysis was performed on heavy polysome-associated mRNAs (three or more ribosomes) from THP1 cells that had been serum-starved for 1 d and that were depleted or not depleted of eIF5B. GO analysis of 86 genes that are underrepresented on polysomes in eIF5B depleted compared with undepleted cells are shown. Only marginally enriched categories with poor statistical significance (Benjamini *P* value 5.7E-2) are observed, suggesting that no particular pathway is specially promoted by eIF5B. This table is related to Figs. 3 and 4 and Fig. S7. BP, biological pathway; CC, cellular component; MF, molecular function.

Other Supporting Information Files

Dataset S1 (XLS)