Supplementary Information

A transcript-specific eIF3 complex mediates global translational control of energy metabolism

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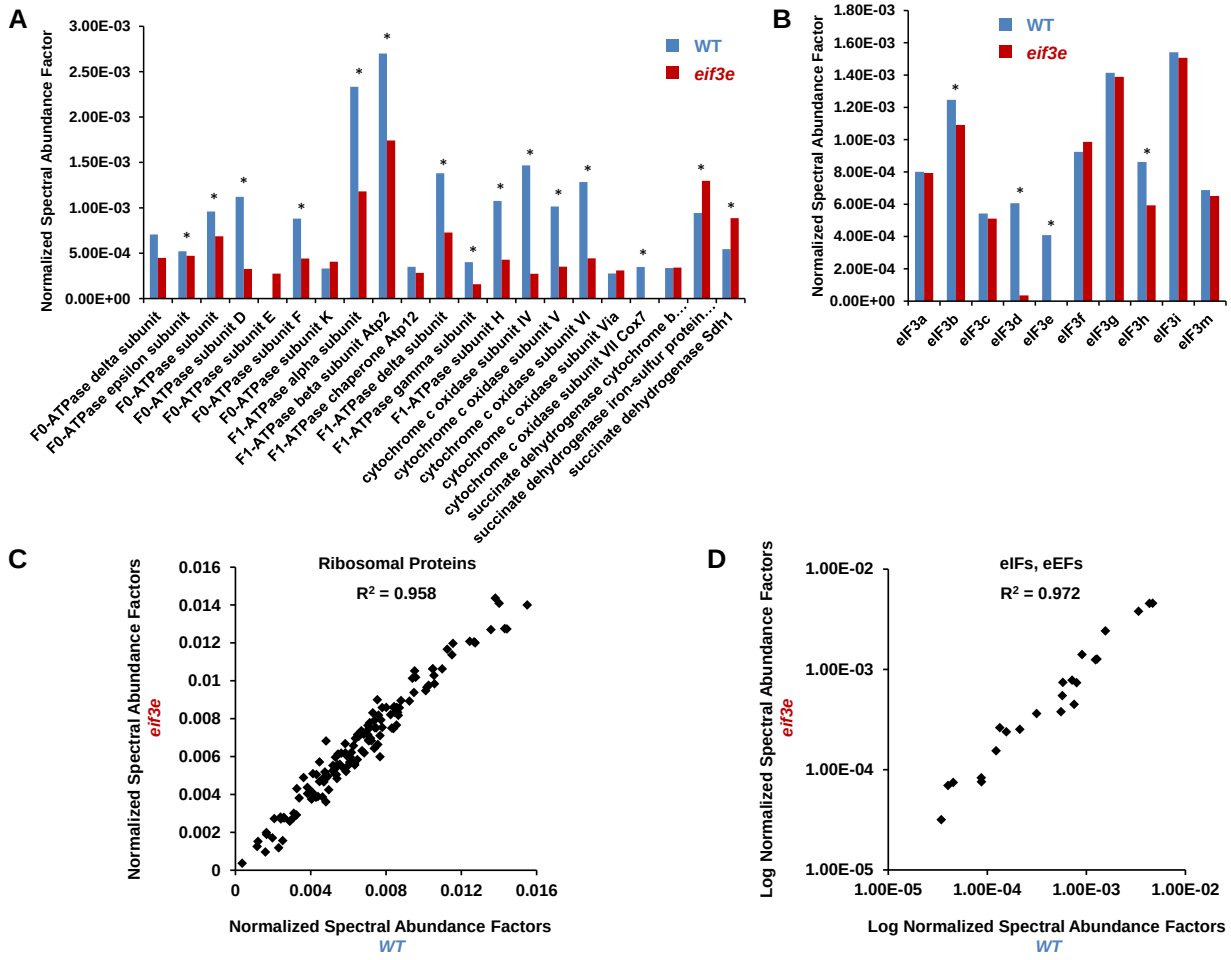

Fig. S1 Abundance of select groups of proteins in 80S complexes (related to Fig. 1 and 2)

A) Abundance of the indicated electron transport chain proteins in 80S complexes isolated from wildtype and *eif3e* deleted cells. Normalized spectral abundance factors (NSAF) (Zybailov et al., 2006), which correct for protein length, were used as quantitative measures. Asterisks indicate significant changes ($p \leq 0.05$).

B) Abundance of the indicated eIF3 subunits in 80S complexes isolated from wildtype cells and from cells deleted for *eif3e* as determined by NSAFs. Note that eIF3 complexes from cells

lacking eIF3e are depleted of eIF3d, whereas other subunits are maintained at roughly stoichiometric levels. Asterisks indicate significant changes ($p \leq 0.05$).

C) NSAFs of ribosomal proteins identified in the 80S complexes purified from wildtype and from cells deleted for *eif3e* are highly correlated, indicating no significant differences in ribosome composition.

D) NSAFs of translation initiation and elongation factors (eIFs, eEFs) identified in the 80S complexes purified from wildtype and from cells deleted for *eif3e* are highly correlated, indicating no significant differences in translation factors.

A Translationally regulated proteins (pSILAC)

Term	Description	Number	Fold of Enrichment	P Value	Benjamini
Down in eif3e					
GO:0015077	Cation transmembrane transporter activity	14	4.67	0.00	0.00
GO:0005746	Mitochondrial respiratory chain	8	7.73	0.00	0.00
GO:0045275	Respiratory chain complex III	5	10.62	0.00	0.03
GO:0045277	Respiratory chain complex IV	3	9.11	0.04	0.47
Up in eif3e					
GO:0008652	Cellular amino acid biosynthetic process	18	4.51	0.00	0.00
GO:0046164	Alcohol catabolic process	10	6.00	0.00	0.00
GO:0006007	Glucose catabolic process	8	6.45	0.00	0.01
GO:0006098	Pentose-phosphate shunt	5	10.76	0.00	0.04
SP_PIR_KEYWORDS	Glycolysis	5	7.02	0.00	0.10
GO:0006099	Tricarboxylic acid cycle	4	5.16	0.04	0.47
GO:0006164	Purine nucleotide biosynthetic process	10	3.69	0.00	0.05

B Steady-state proteins

Term	Description	Number	Fold of Enrichment	P Value	Benjamini
Down in eif3e					
GO:0042254	Ribosome biogenesis	75	5.03	0.00	0.00
GO:0005746	Mitochondrial respiratory chain	6	4.35	0.01	0.18
GO:0045277	Respiratory chain complex IV	3	6.83	0.07	0.64
Up in eif3e					
GO:0009309	Amine biosynthetic process	42	7.33	0.00	0.00
GO:0006006	Glucose metabolic process	15	7.46	0.00	0.00
GO:0046164	Alcohol catabolic process	15	6.94	0.00	0.00
glycolysis	Glycolysis	8	8.67	0.00	0.00
GO:0006099	Tricarboxylic acid cycle	8	7.95	0.00	0.00
GO:0019321	Pentose metabolic process	6	7.02	0.00	0.02
GO:0009126	Purine nucleoside metabolic process	6	6.63	0.00	0.03
GO:0006098	Pentose-phosphate shunt	5	8.29	0.00	0.04
GO:0006537	Glutamate biosynthetic process	3	8.52	0.04	0.36

C Steady-state mRNA

Term	Description	Number	Fold of Enrichment	P Value	Benjamini
Down in eif3e					
GO:0006364	rRNA processing	11	2.12	0.03	1.00
GO:0033554	Cellular response to stress	29	1.40	0.05	0.99
GO:0055085	Transmembrane transport	15	1.68	0.06	0.97
Up in eif3e					
GO:0009228	Thiamin biosynthetic process	4	15.77	0.00	0.30
GO:0006865	Amino acid transport	5	4.06	0.03	0.69
GO:0033554	Cellular response to stress	34	1.36	0.05	0.78
GO:0016021	Integral to membrane	45	1.29	0.05	0.93

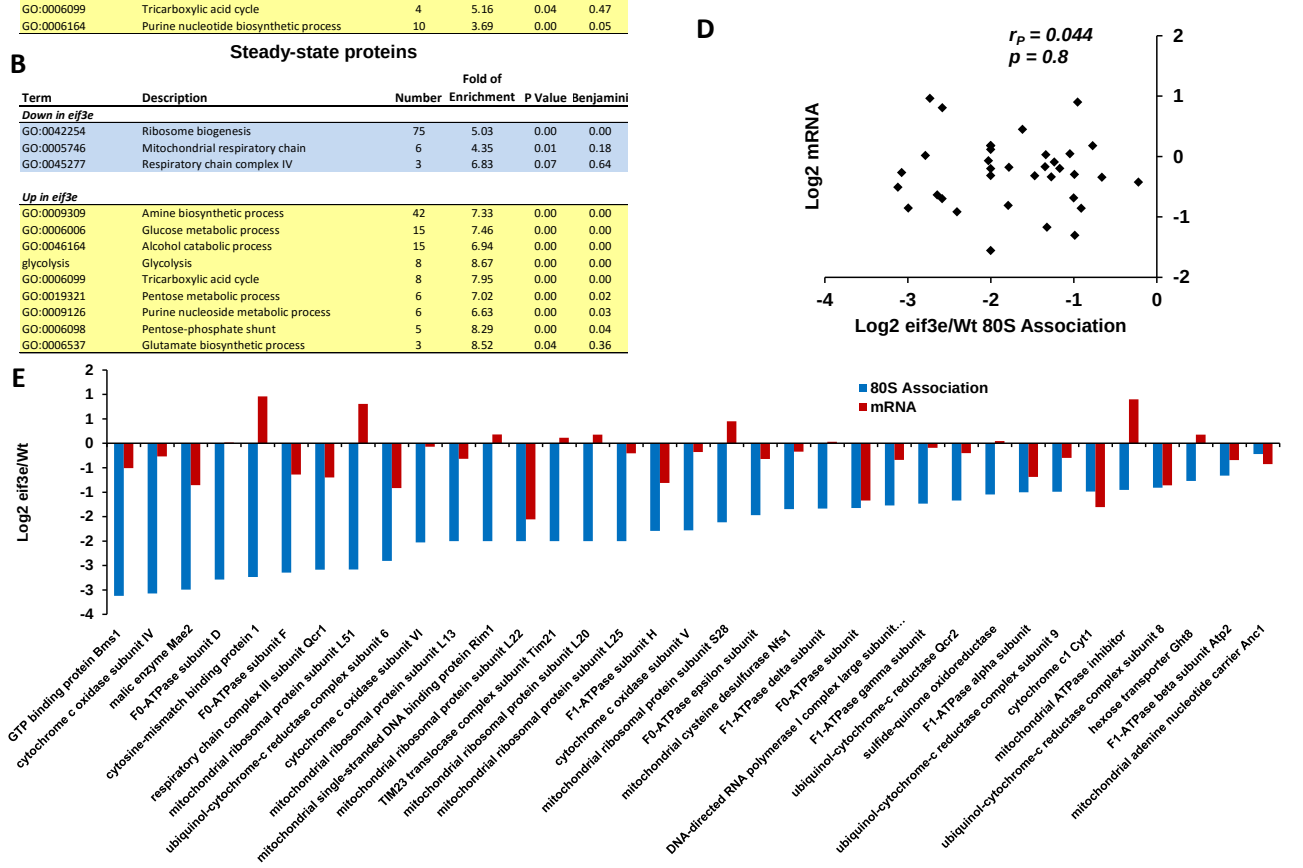

Fig. S2 Gene Ontology (GO) term enrichment and mRNA-protein correlations (related to Fig. 2)

Selected GO terms enriched in the translatomic (pSILAC) (**A**), steady-state proteomic (**B**), and transcriptomic (**C**) datasets. P values and corrected p values (Benjamini) are indicated.

D) Correlation between changes in the association of 35 mitochondrial proteins with 80S complexes and their mRNA levels in the absence of *eif3e*. Translation rates are averages of two replicates. Pearson coefficient and p value of an ANOVA regression analysis are indicated.

E) Log2 changes in 80S association of 35 mitochondrial proteins and their corresponding log2 mRNA changes in the absence of *eif3e*.

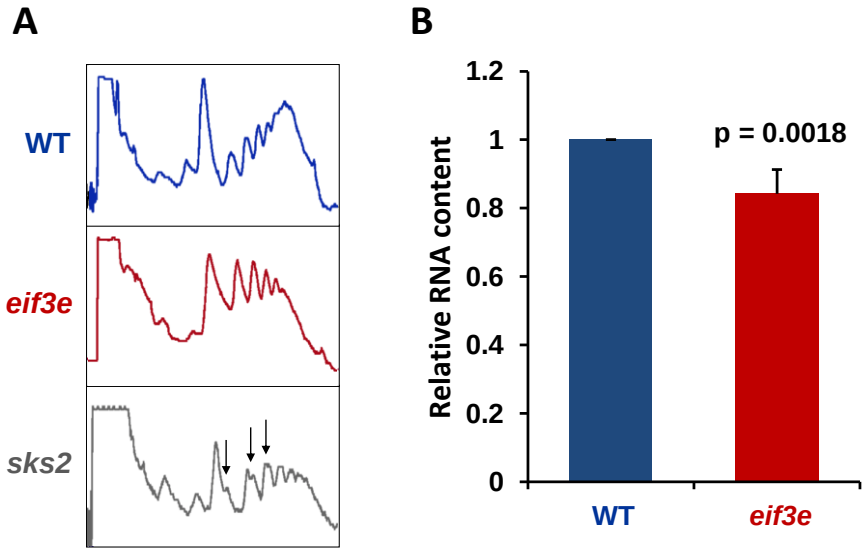

Fig. S3 Effect of deletion of *eif3e* on ribosome biogenesis (related to Fig. 3)

A) Lysate of the indicated strains was fractionated by sucrose density gradient centrifugation, and elution profiles were obtained. Unlike the *sks2* deletion strain (grey) which has an established ribosome biogenesis defective, eif3e deleted cells do not show ribosome halfmers (arrows), suggesting that they do not suffer from a primary defect in ribosome biogenesis.

B) Relative amounts of total RNA extracted from wild-type (WT) and *eif3e* deleted cells normalized to cell mass (OD). Plotted are averages from 5 independent RNA extractions. A t-test was used to determine the p value of the difference between WT and mutant. Error bars represent standard deviations.

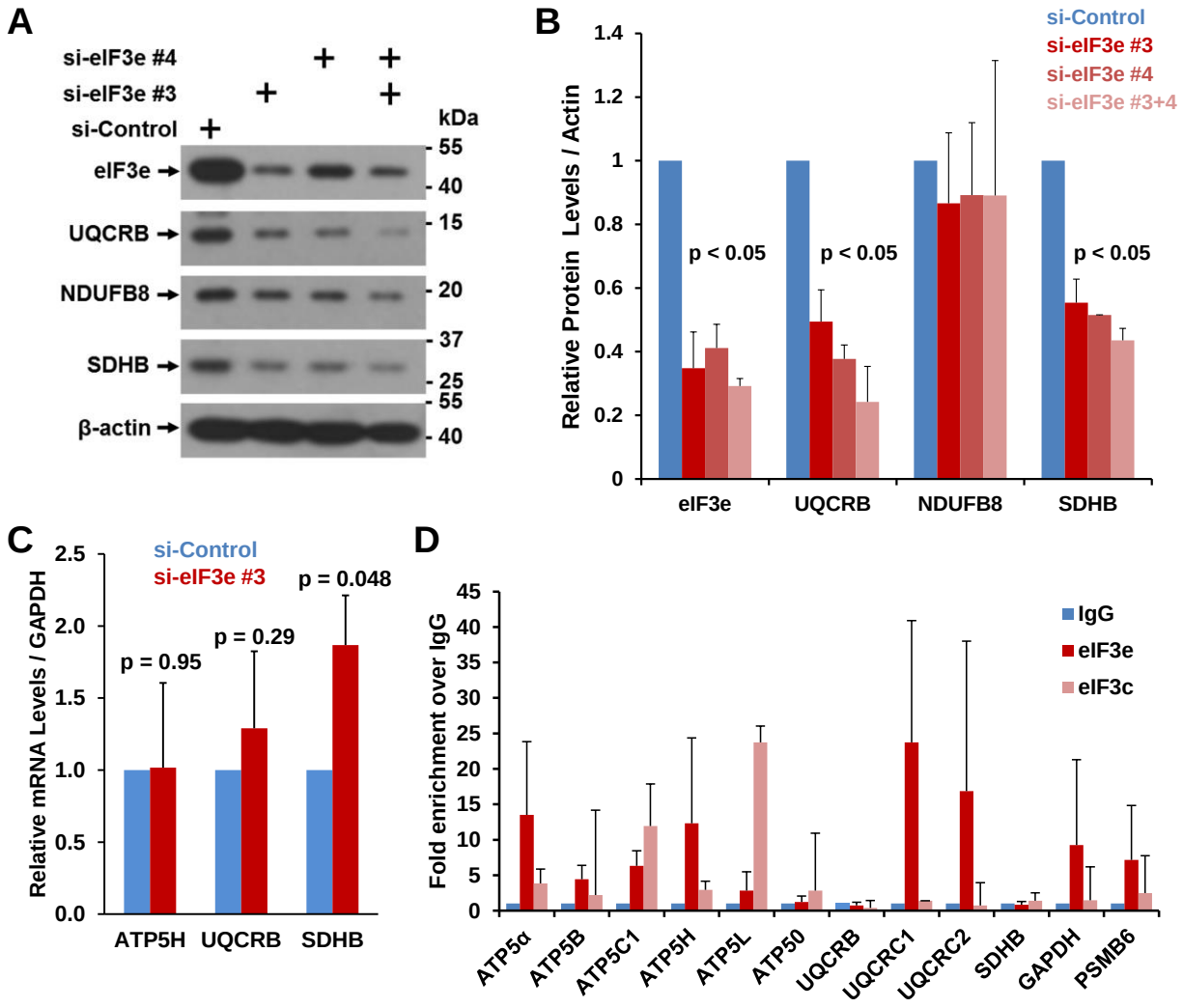

Fig. S4 Effect of knockdown of eIF3e on ETC proteins in MCF10A cells and eIF3-mNA interactions in HeLa cells (related to Fig. 6)

A) eIF3e was knocked down in non-tumorigenic MCF10A cells, followed by determination of the levels of the indicated ETC proteins by immunoblotting.

B) Blots from biological replicates were quantified with the Licor Image Studio Lite package and results corrected for the loading reference were plotted in a bar graph. Error bars indicate standard deviations. Statistical significance was assessed with a t-test assuming two-tailed distribution and unequal variance. P values for differences to the si-Control values are indicated.

C) eIF3e was knocked down in MCF10A cells, followed by determination of the levels of the indicated mRNA by quantitative RT-PCR relative to GAPDH mRNA as a reference. Averaged data from five (ATP5H, UQCRB) or three (SDHB) biological replicates were plotted in a bar graph. Error bars indicate standard deviations. Statistical significance was assessed with a t-test assuming two-tailed distribution and unequal variance. P values for differences to the si-Control values are indicated.

(**D**) eIF3 complexes were immunopurified from HeLa cells using antibodies against eIF3e and eIF3c as indicated. eIF3-associated mRNA was extracted and quantified by q-PCR. The graph shows fold change in the enrichment relative to the IgG control. Data represent averages of 4 independent experiments, and error bars indicate standard deviations.

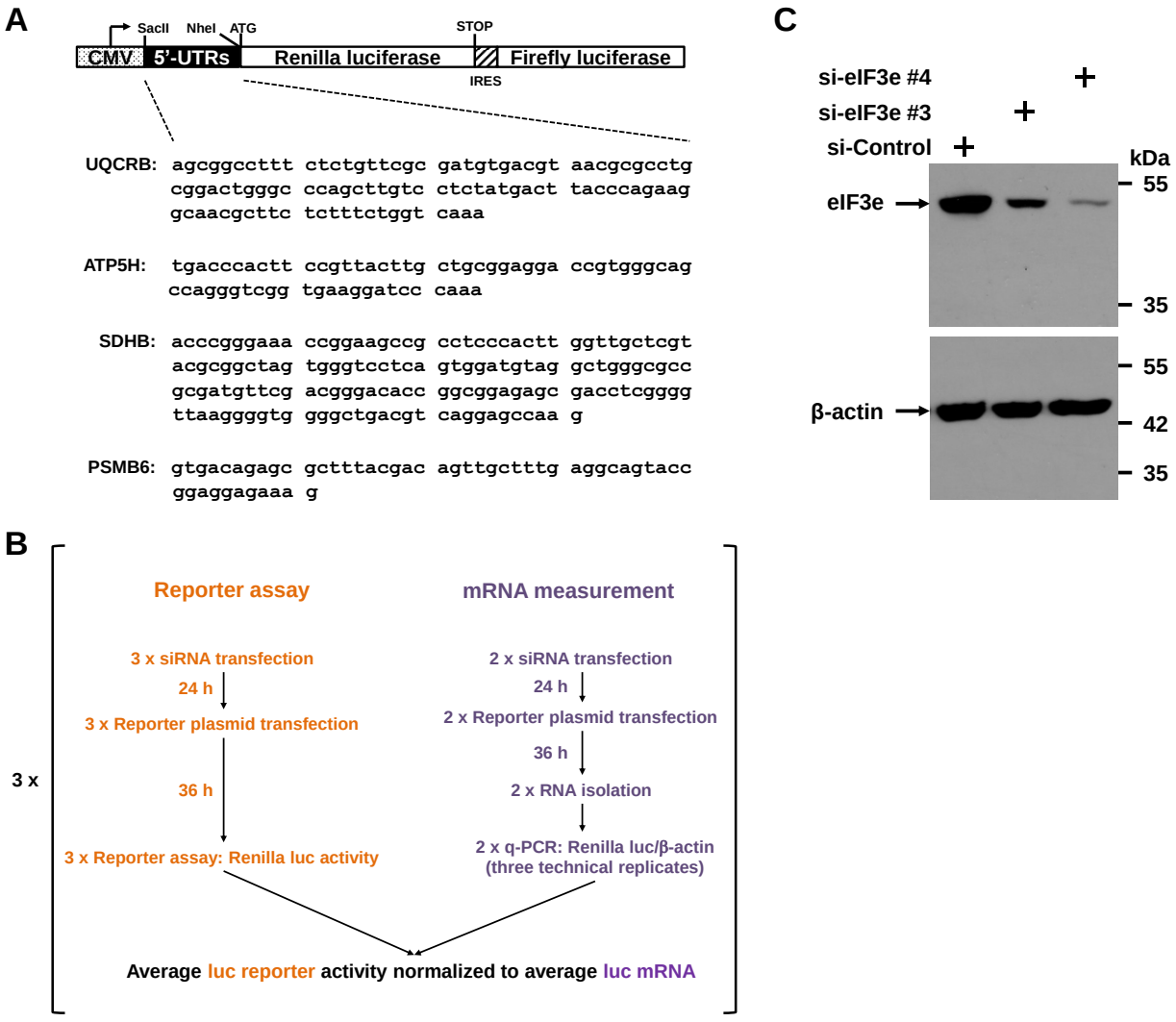

Fig. S5 Luciferase reporter assay (related to Fig. 6)

A) Schematic of the renilla luciferase reporter constructs used. The sequences of the 5'-UTRs cloned into the reporter constructs according to the genes' Genbank entries.

B) Workflow of the reporter assay. Parallel transfections were carried out in replicates as indicated for the reporter assays and measurement of renilla mRNA. The entire workflow was performed independently three times, and the data of all replicates were averaged in the graph in Fig. 6C.

C) Confirmation of eIF3e knockdown efficiency in a representative reporter experiment. Cell lysate used for luciferase assays was subjected to immunoblotting with eIF3e antibodies. Actin is shown as a reference.

Supplemental Materials and Methods (related to Experimental Procedures)

Yeast strains

The genotypes of the strains and the figures in which they were used are summarized in Supplementary Table 1. The *sks2* gene was replaced with the NAT gene conferring resistance to nourseothricin. S. pombe strains were maintained in standard yeast extract (YES) or Edinburgh Minimal Media (EMM) unless otherwise noted.

Supplementary Table 1

Name	Genotype	Used in Fig.
JSS4	h90 ade6-M210 ura4-D18 leu1-32	1A, 1B, 2, 3, 4A-D, 5, S1A, S2, S3, S4
JSS5	h90 eif3e/int6/yin::kanMX6 ade6-M210 ura4-D18 leu1-32	1A, 1B, 2, 3, 4A-D, 5, S1A, S2, S3, S4
JSS22	h90 eif3d/moe1::ura4 ade6-M210 ura4-D18 leu1-32	1a, 4A-D, 5
972	h-	3A
DC024	h- eIF3d/moe1::kanMX6	3A
DC026	h- eIF3h/tif38::kanMX6	3A
DC027	h+ eIF3j/hcr1::kanMX6	3A
AZ85	h- coq4::kanMX6	3A
DS448/2	h- ura4-D18 leu1-32 ade6-704	S1B, 4E
C617/1	h- eif3m-CBP-TEV-5proA::kanMX6 leu1-32 ura4-d18 ade6-704	S1B, 4E
C648	h+ eif3e-CBP-TEV-5proA::kanMX6 leu1-32 ura4-d18 ade6-704	S1B, 4E
sks2Δ	h90 sks2::natMX6 ade6-M210 ura4-D18 leu1-32	S4

Sucrose density gradient profiling

10-50% (w/v) sucrose gradients were prepared in polysome lysis buffer (20 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 100 µg/ml cycloheximide). The same buffer supplemented with 200 µg/ml heparin and 1 mM PMSF was used for cell lysis. Lysate prepared from exponentially growing cells (OD_{600} 0.5-1.00) was prepared by bead lysis (FastPrep device, 20 seconds at speed setting 6), cleared by centrifugation, and 18 OD_{260} were loaded onto a sucrose gradient. Gradients were centrifuged in a Beckman SW40Ti rotor at 39.000 rpm for 150

min at 4ºC. Gradients were fractionated by upward displacement with 55% (w/v) sucrose, and fractions were collected.

For immunoblotting, fractions were mixed with SDS sample buffer. For RNA isolation, collected fractions were precipitated in 1 volume of isopropanol at -80ºC, followed by purification via the RNeasy Kit (Quiagen). For RT-PCR of *ght5*, RNA from all fractions across the sucrose density gradient was precipitated in isopropanol and purified using the RNeasy Kit (Quiagen). DNA was digested using 1.5 U of DNase I (Thermo) for 20 min, and the RNA was reverse transcribed to cDNA using Superscript III reverse transcriptase (Life Technologies) with a mix of oligo(dT) and random hexamer primers and in the presence of RNase inhibitors. The *ght5* cDNA was amplified with forward primer ATTGGTGCTCTTTGGCAATC and reverse primer TTGAACACCACGGGATTTTT using Crimson *Taq* DNA polymerase (New England BioLabs).

Affinity purification of eIF3e-ProA and eIF3m-ProA

Protein A-tagged eIF3e and eIF3m were purified from pooled monosomal and polysomal fractions obtained after polysome profiling. Dynabeads® M-280 Tosyl activated (Invitrogen) were washed and coupled with IgG in 0.1 M Na-phosphate buffer pH 7.4 overnight at room temperature. Beads were washed in PBS with 0.5% (w/v) BSA, followed by additional washes in PBS with 0.1% (w/v) BSA. Beads were added to pooled monosomal or polysomal sucrose gradient fractions that were diluted with 1 volume of IP lysis buffer (50 mM Tris pH 7.4, 140 mM NaCl, 0.5% Triton X-100). After 1h, the supernatant was removed, and beads were washed 3 times in IP lysis buffer, followed by elution with 1% (w/v) SDS. Samples were analyzed by immunoblotting.

Preparation of 80S complexes for LC-MS/MS analysis

110 ml yeast cultures of wild-type and *eif3e* deleted cells in exponential growth phase were collected in 100 µg/ml cycloheximide and lysed in a buffer containing 20 mM Tris pH 8, 140 mM KCl, 5 mM $MgCl_2$, 100 µg/ml cycloheximide, 1% Triton X-100, 200 µg/ml heparin, and 1 mM PMSF using a Fastprep device. An amount equivalent to 40 OD260 of lysate was digested with 750 U of RNAse1 (Ambion) for 30 minutes at room temperature. The reaction was stopped by the addition of 300 U SuperaseIn (Ambion), and lysates were loaded onto 25% sucrose cushions in polysome lysis buffer without Triton X-100, heparin or PMSF, but additionally supplemented with 0.5 mM DTT and 20 U/ml SuperaseIn. Samples were centrifuged at 45.000 rpm for 4.5 h in an SW55 rotor, and ribosomal pellets were resuspended in A) SDS sample buffer (60 mM Tris-HCl pH 6.8, 5% ß-mercaptoethanol, 2% SDS, 10% glycerol) or B) Urea buffer (50 mM ammonium bicarbonate pH 7.5, 8 M urea). The samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Brill et al., 2009).

Briefly, samples were digested with trypsin using a modified Filter-Aided Sample Preparation (FASP) method (Wiśniewski et al., 2011). Following protein reduction and alkylation, peptides were digested with trypsin, desalted, and analyzed by high-resolution, high-accuracy LC-MS/MS, consisting of a Michrom Magic C18 column, a low-flow ADVANCED Michrom MS source, and a LTQ-Orbitrap XL. The LC-MS/MS raw data was analyzed using Integrated Proteomics Pipeline (IP2) version 1.01 with the ProLucid algorithm as the algorithm for protein identification, searching the SPOMBE_V2_21 fasta protein database. Precursor mass tolerance was set to 50 ppm for the first search and then filtered with 5 ppm with DTASelect. Carbamidomethylation of cysteine and oxidation of methionine were searched as variable

modifications. Enzyme was set to trypsin in a semi-specific mode and a maximum of two missed cleavages was allowed for searching. The target-decoy-based false discovery rate (FDR) filter for spectrum and protein identification was set to 2%. The search results were sorted, filtered using DTASelect and statistically analyzed by IP2 label-free Protein Identification STAT COMPARE tool with NSAF. The data were submitted to the Pride database for public access (PXD004147).

For analysis of newly synthesized components, wild-type and *eif3e* deleted cells were switched from YES to EMM growth media containing ^{14}N-$NHCl_4$ as the sole nitrogen source for 4 h. Purification of 80S complexes and LC-MS/MS analysis was performed as described above. Statistically significant differences in the rate of synthesis of 80S-associated proteins between wild-type and *eif3e* mutant cells were derived from calculating ^{15}N-WT and ^{15}N-*eif3e* protein ratios from ^{15}N-labeled peptides using IP2 software. The data were submitted to the Pride database for public access (PXD004146).

Quantitative proteomic profile

Wild-type and *eif3e* deleted cells were grown to exponential phase in YES medium followed by bead lysis in a Fastprep device. After lysis, beads were collected in SDS sample buffer (60 mM Tris-HCl pH 6.8, 5% ß-mercaptoethanol, 2% SDS, 10% glycerol). Samples were analyzed by on-line two-dimensional reversed phase chromatography coupled to mass spectrometry (2D-bRP-RP-MS/MS).

Prior to digestion, protein concentration was determined using bicinchoninic acid (BCA) protein assay (Pierce, Waltham, MA). Samples were digested using a modified Filter-aided Sample Preparation (FASP) protocol (Wiśniewski et al., 2011). In brief, each sample was

transferred to a 10 kDa molecular weight cutoff filter (Millipore, MA, USA). All following centrifugations were carried out at 14000 rpm in a benchtop microfuge. First, the samples were washed 3x with 300 µL of UA buffer (8 M urea, 50 mM ammonium bicarbonate), and cysteine disulfide bonds were reduced with 5 mM dithiothreitol (DTT) at 30°C for 60 min followed by cysteine alkylation with 15 mM iodoacetamide (IAA) in the dark at room temperature for 30 min. Following alkylation, the samples were washed 2x with 300 µL of UB buffer (1 M urea, 50 mM ammonium bicarbonate). The samples were finally subjected to overnight digestion with trypsin (Promega, Madison, WI). Finally, the peptides were eluted off the filter by centrifugation at 14,000 rpm. In addition, the filter was washed twice with 200 µL of 15% acetonitrile to increase peptide recovery. The digested samples were partially dried to approximately 50% of the total volume, and desalted using a C18 TopTip (PolyLC). Finally, all samples were dried on a SpeedVac system.

Desalted samples were reconstituted in 1.5% acetonitrile in 100mM ammonium formate pH ~10. A total of 10 ug were then loaded onto the first dimension column, XBridge BEH130 C18 NanoEase (300 µm x 50 mm, 5 µm) using a 2D nanoACQUITY Ultra Performance Liquid Chromatography (UPLC) system (Waters corp., Milford, MA) equilibrated with solvent A (20 mM ammonium formate, pH 10, first dimension pump) at 2 µL/min. The first fraction was eluted from the first dimension column at 10.3% of solvent B (100% acetonitrile) for 4 min and transferred to the second dimension Symmetry C18 trap column 0.180 x 20 mm (Waters corp., Milford, MA) using a 1:10 dilution with 99.9% second dimensional pump solvent A (0.1% formic acid in water) at 20 µL/min. Peptides were then eluted from the trap column and resolved on the analytical C18 BEH130 column 0.075 x 100 mm, 1.7um particles (NewObjective, MA) at low pH by increasing the composition of solvent B (100% acetonitrile) from 3 to 35% over 44

min at 0.4 μL/min. Subsequent fractions were carried with increasing concentrations of solvent B. The following 14 first dimension fractions were eluted at 11.5, 12.5, 13.3, 14.1, 14.9, 15.7, 16.5, 17.4, 18.4, 19.6, 21.0, 22.8, 26.7, and 65% solvent B. The analytical column outlet was directly coupled to an Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific) operated in positive data-dependent acquisition mode. MS1 spectra were measured with a resolution of 60,000, an AGC target of 10^6 and a mass range from 350 to 1400 m/z. Up to 10 MS2 spectra per duty cycle were triggered, fragmented by collision-induced dissociation, and acquired in the ion trap with an AGC target of 10^4, an isolation window of 2.0 m/z and a normalized collision energy of 35. Dynamic exclusion was enabled with duration of 30 sec.

All mass spectra from were analyzed with MaxQuant software version 1.5.0.25 (Cox and Mann, 2008) in identical fashion otherwise noted. MS/MS spectra were searched against the Pombase protein sequence database ASM294 (version 2.23). Precursor mass tolerance was set to 20 ppm for the first search where initial mass recalibration was completed and to 4.5 ppm for the main search. Product ions were searched with a mass tolerance 0.5 Da. The maximum precursor ion charge state used for searching was 7. Carbamidomethylation of cysteine was searched as a fixed modification, while oxidation of methionine was searched as variable modifications. Enzyme was set to trypsin in a semi-specific mode and a maximum of two missed cleavages was allowed for searching. The target-decoy-based false discovery rate (FDR) filter for spectrum and protein identification was set to 1%. We required 1 or more unique/razor peptides for protein identification and a ratio count of 1 or more for protein quantification. Second peptide mode of MaxQuant software was also enabled. Features were matched between runs using a maximal ‘match time window’ of 0.7 min. The data were submitted to the Pride database for public access (PXD004137).

Pulsed SILAC

Wild-type and *eif3e* deleted cells were grown to exponential phase in YES medium, washed and switched to EMM containing 30 mg/L isotopically labeled $^{13}C6$ $^{15}N2$ lysine (K8). Cell lysate was prepared after 2 hours and analyzed by 2D-bRP-RP-MS/MS on an Orbitrap Velos Pro mass spectrometer as described above. MaxQuant was used for quantifying K8-labeled peptides. Statistically significant differences in the rate of synthesis of individual proteins between wild-type and *eif3e* mutant cells were derived from calculating K8-WT and K8-*eif3e* protein ratios from K8-labeled peptides. The data were submitted to the Pride database for public access (PXD004138).

RNA seq analysis

Total RNA was extracted by phase separation using RNA-Bee^{TM} (Amsbio) and chloroform, and precipitated in isopropanol. RNA was further purified using the RNeasy Mini Kit (Qiagen), performing an on-column DNase digestion. The quality of total RNA was assessed by the Agilent Bioanalyzer (Agilent Technologies). Total RNA samples were treated with RiboMinus Eukaryotic kit (Invitrogen) to deplete rRNA. The rRNA-depleted sample was subjected to enzymatic fragmentation using RNase III from the SOLiD Total RNA-Seq kit. The purified fragmented RNA was then ligated with SOLiD adaptors on both ends and served as templates for cDNA synthesis. The cDNA libraries were constructed and purified twice using AMPure beads to ensure complete capture and size-selection of desired cDNA. The cDNA was PCR amplified and sequenced on the SOLiD 4 platform. Reads generated from RNA seq were processed following similar steps as for ribosome profiling except that the trimming step was omitted.

Bowtie was used first to align the reads to tRNA and rRNA sequences, and unaligned reads were aligned to the genome using TopHat. The Expectation-Maximization approach was used to estimate transcript abundance (Jiang and Wong, 2009). Normalized abundance (RPKM) was calculated for each transcript (Mortazavi et al., 2008). R package edgeR was used to call the differential expression results (Robinson et al., 2010). The RNA-seq data were submitted to the Gene Expression Omnibus repository (accession number GSE80349).

Measurement of oxygen consumption

Oxygen consumption was measured as described before (Zuin et al., 2008), with some modifications. Briefly, cell cultures were first grown in rich media to reach stationary phase, then diluted into fresh media at a concentration of ~10^5 cells/ml. Growth at 30 °C continued for approximately 15-17 hr until cultures reached a final OD_{600} of 0.5. Cell pellets were washed once with fresh EMM, and cell suspensions adjusted to a final OD_{600} of 1 (~ 10^7 cells/ml) were prepared for recording. Oxygen consumption from 1 ml cell suspensions was measured using a Hansatech Oxygraph (Hansatech), with readings being recorded over a duration of 15 minutes.

Metabolomic profiling by LC-MS and MetaboAnalyst analysis

Metabolomic analysis was performed as described previously (Pluskal et al., 2010a). Briefly, wild-type and *eif3e* deleted cells from three independent liquid cultures cultivated in the YES medium at 30 °C (~10^8 cells/sample) were collected by vacuum filtration and immediately quenched in 25 ml methanol (-40 ºC). Cells were harvested by centrifugation at -20 ºC and constant amounts of internal standards (10 nmol of HEPES and PIPES) were added to each sample. Disruption was performed using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan).

Proteins were removed by centrifugal filtration on an Amicon Ultra 10-kDa cut-off filter (Millipore, Billerica, USA) and samples were concentrated by vacuum evaporation. Finally, each sample was re-suspended in 40 µl of 50% acetonitrile and 1 µl was used for liquid chromatography mass spectrometry (LC-MS) analysis on a Paradigm MS4 HPLC system (Michrom Bioresources, Auburn, USA) coupled to an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, USA). LC separation was performed on a ZIC-pHILIC column (Merck SeQuant, Umeå, Sweden; 150 × 2.1 mm, 5 µm particle size). Acetonitrile (A) and 10 mM ammonium carbonate buffer, pH 9.3 (B) were used as mobile phase, with gradient elution from 80% A to 20% A in 30 min at 100 µl/min flow rate. Peak areas of metabolites of interest were integrated using the MZmine 2.14.2 software (Pluskal et al., 2010b) and normalized by the peak areas of the spiked internal standards.

The LC-MS data was filtered to remove peak area signals lower than 2, compounds of unknown structure and duplicates, leaving 265 known compounds out of the initial ~8050 data points. The filtered data were loaded into MetaboAnalyst (http://www.metaboanalyst.ca/), normalized by sum, log transformed and autoscaled (mean-centered and divided by standard deviation). Significant changes in 90 metabolites between wildtype and *eif3e* deleted cells were identified by T-test ($p<0.05$, fold change cut-off 1.2). The compound list was used for Integrated Pathway Analysis in MetaboAnalyst along with a list of 521 proteins that were significantly changes by deleting *eif3e* as determined by our proteomic profile. Since this functionality of MetaboAnalyst can only handle mammalian protein sets, we used the predicted human orthologues of the S. pombe proteins. Pathway enrichment analysis was done by hypergeometric test, topology analysis was based on degree centrality, and a combined gene-metabolite mode

was used. Enrichment and topology significance was expressed as Z scores of –log P and topology values (Xia and Wishart, 2010).

Determination of survival rates of chronologically aged cultures

Cell viability in stationary phase was assessed essentially as described by Roux et al. (Roux et al., 2006). In brief, overnight pre-cultures of wildtype cells and cells deleted for *eif3e* and *eif3d* were prepared in YES medium at 30 ^{o}C. From these pre-cultures, duplicate 10 ml cultures were inoculated at an OD_{600} of 0.1. The cultures were grown to stationary phase (OD_{600} = 9 - 10), which was considered as day 0 of the experiment. 200 cells removed from these cultures on 5 consecutive days were plated onto YES plates, incubated at 30 ^{o}C for 7 days, and the total number of colonies was counted. The colony numbers at day 0 was set to represent 100% survival. For the viability test based on the exclusion of propidium iodide as an indicator of metabolic activity, aliquots of the culture described above were centrifuged, washed twice with PBS, and incubated with 5 μg/ml of propidium iodide for 30 min on ice in darkness. 10,000 cells were analyzed by flow cytometry using the FL2 channel. The percent of propidium iodide negative cells was set to represent 100% survival at day 0.

Relative quantification of protein A-tagged eIF3 subunits by immunoblotting

Strains harboring alleles of *eif3e* and *eif3m* modified with protein A epitope tags (Zhou et al., 2005) were shifted from YES media containing 3% glucose to medium containing 0.08% glucose. Cell lysates were loaded on SDS-PAGE gels in duplicates and probed with anti-protein A antibodies to detect eIF3e and eIF3m, respectively. Bands obtained with anti-PSTAIR antibodies (Santa Cruz) were used as loading reference. The antibody recognizes a band of ~34

kDa corresponding to Cdc2p. The antibody also reacts non-specifically with a series of other proteins that are shown in the bottom panels of Fig. 4E. Because there is no known protein that can serve as a universal "loading control" under any condition, the signals obtained for eIF3 subunits were normalized to the signal of all proteins stained with the PSTAIR antibody. The analysis of quantitative proteomic datasets has shown that small differences (such as obtained for eIF3 subunits in the experiment in Fig. 4E) can be reliably quantified when normalized to the signal of many unchanged proteins (thousands in the case of proteomic datasets). The approach of normalizing to total PSTAIR antibody signal is to adapt the experience from large scale proteomics to the Western blotting format. Blots from biological replicates were quantified with the Licor Image Studio Lite package and results corrected for the loading reference were plotted. Statistical significance was assessed with a t-test assuming two-tailed distribution and unequal variance. Asterisks indicate $p < 0.05$.

eIF3e knockdown studies

MCF7 cells (ATCC) were maintained in DMEM (Hyclone) with 10% FBS and MCF10A cells (ATCC) were maintained in DMEM/F12 (Gibco) supplemented with 5% FBS, 10 ng/ml EGF,10 ug/ml insulin, 5 ug/ml Hydrocortisone and 1% penicillin-streptomycin in 5% CO_2 at 37℃.
For knockdown studies, cells were grown to 30% - 40% confluence in 12-well plates.100 nM of siRNA was transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. 10 μl Lipofectamine 2000 was diluted in 250 μl Opti-MEM media, mixed gently, and incubated for 5 min at room temperature. siRNA was diluted in 250 μl Opti-MEM media (final concentration of siRNA when added to the cells was 100 nM) and mixed gently. siRNA diluted Lipofectamine 2000 was incubated for 20 min at room temperature and the mixture was added to

cells. After 4 – 6 hours, media was changed and cell lysate was obtained 72 hours later and analyzed by immunoblotting. siRNAs were purchased from Qiagen. siRNA target sequence for eIF3e were as follows: siRNA#3(SI02662499): 5'-CCCAAAGGTCGCGATAATATT-3', siRNA#4(SI02661981):5'-AAGCTGGCCTCTGAAATCTTA-3'. The levels of eIF3e and ETC proteins were examined by immunoblotting using the following antibodies: eIF3e (Abcam, ab36766,1:5000), ATP5H (Thermo-Fisher Scientific, PA5-23694,1:500), UQCRB (Abcam, ab122827,1:200), total OXPHOS Rodent WB Antibody Cocktail (Abcam, ab110413,1:250), actin (Sigma, A5441). Blots were probed with horse radish peroxidase coupled secondary antibodies and signals were detected by chemiluminescence on X-ray films. Low exposure blots were scanned and quantified with Image Studio Lite 5.2 (Licor). Signals were normalized to the actin reference and plotted in bar diagrams. Statistical analysis of duplicate experiments was performed in Excel. Statistical significance was assessed with a t-test assuming two-tailed distribution and unequal variance.

Luciferase report assay

Reporter vector pcDNA3-rLuc-IRES-fLuc was obtained from Addgene (plasmid id# 45642). Upon removal of the 5'UTR and T7 promoter, a unique SacII site was introduced downstream of the transcription start site and upstream of a NheI site located next to the luciferase start codon. The 5'UTR sequences of ATP5H, PSMB6, SDHB, UQCRB (Fig. S5A) were obtained as oligonucleotides with a SacII site at the 5' and a NheI at the 3' end. The primers were annealed and cloned into pcDNA3-rLuc-IRES-fLuc-5'UTR^{-} cleaved with SacII and NheI.

eIF3e was knocked down in MCF10A cells using siRNA#3(SI02662499): 5'-CCCAAAGGTCGCGATAATATT-3' and siRNA#4(SI02661981):5'-

AAGCTGGCCTCTGAAATCTTA-3' obtained from Qiagen. Non-specific siRNA was obtained from the Functional Genomics facility of Sanford Burnham Prebys Medical Discovery Institute. For siRNA transfections, six pmol siRNA was mixed with 100 µl of Opti-MEM and 1 ul of Lipofectamine RNAiMAX reagent (Invitrogen) and incubated for 30 mins. 60,000 MCF10A cells in 500 µl media were placed into each well of a 24-well plate. The media was changed to MCF10A culture media after 8 hours. After 24 hours, luciferase reporter plasmids were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The media was changed to MCF10A media after 4-5 hours. 36 h after transfection, luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega). Cells were lysed in 100 µl of 1x passive lysis buffer for 30 mins, and 30 µl of the lysate was used to measure the reporter activity in a luminometer.

For normalization of reporter activity to luciferase mRNA expressed in cells from the various plasmids, q-PCR was carried out. 36 hours after transfection, mRNA was extracted from wells prepared in parallel to the reporter assay using the RNesy mini kit (Qiagen) according to the manufacturer's protocol. cDNA was synthesized from 500 ng total RNA per reaction using M-MuLV reverse transcriptase (New England) according to manufacturer's protocol. cDNA was diluted 5 times, and 3 µl of the diluted cDNA was used for q-PCR. Real time PCR samples contained 10 µl FastStart Universal SYBR green master mix (Roche), 0.35 µM of each primer (Table S2), and 3 µl of the diluted cDNA. Reactions were processed for one cycle at 94°C for 10 min, followed by 40 cycles at 94°C for 20 s and at 68°C for 60 s in a Stratagene 3000X real time PCR machine. Relative expression was measured with the 2ΔCt method using β-actin as the reference transcript (Livak and Schmittgen, 2001). Each experiment was repeated 3 times (Fig. S5B), and statistical significance was calculated with a Student's *t*-test.

RNA immunoprecipitation and q-PCR

The RNA-IP of eIF3 subunits was optimized for two cell lines: MCF7 and HeLa cells. Two 150 mm plates of MCF7 cells were washed with cold PBS, scraped into 15 ml cold PBS, and pelleted by centrifugation at 1,200 rpm for 10 min at 4℃. The pellet was lysed in 1 ml IP lysis buffer (50 mM Hepes-OH, pH 7.5, 150 mM KCl, 2 mM EDTA, 0.5% NP-40, 0.5 mM DTT, 1 x complete EDTA-free proteinase inhibitor cocktail tablet (Roche), 100U/ml RNase inhibitor (Promega). The cell suspension was vortexed for 10 s and incubated on ice for 15 min, followed by centrifugation at 20,000 rpm at 4 ℃ for 2min. The supernatant was rotated with 400 U/ml RNase-free DNAse I (Roche) for 30min at 4 ℃ and diluted into a total volume of 2.5 ml with lysis buffer.

2 μg of eIF3e antibody (Bethyl A302-985A), eIF3c antibody (Bethyl A301-761A), or normal rabbit IgG (Santa Cruz, sc-2027) were added, followed by incubation at 4 ℃ overnight. 25 μl of Bio-Rad magnetic protein G beads (Cat. No. 161-4023) were added to each sample and incubated with rotation for 2 hours at 4 ℃. Beads were collected and washed five times in high-salt buffer (50 mM Hepes-OH, pH 7.5, 150 mM KCl, 2 mM EDTA, 0.5% NP-40, 0.5 mM DTT). After the final wash, beads were resuspended in 200 ul elution buffer (1% SDS, 1% $NaHCO_3$, 400 U/ml RNase inhibitor).

For RNA-IP from HeLa cells, two 150 mm plates of cells were washed with cold PBS, scraped into 15 ml cold PBS, and pelleted by centrifugation at 1,200 rpm for 10 min at 4℃。The pellet was lysed in 1 ml IP2 lysis buffer (10 mM Hepes-OH, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 10% NP-40, 1x complete EDTA-free proteinase inhibitor cocktail tablet (Roche), and 100U/ml RNase inhibitor (Promega)). Following

suspension by vortexing for 10 s, samples were incubate on ice for 10 min and centrifuged at 20,000 rpm for 2 min at 4 ℃. The lysate was rotated with 400 U/ml RNase-free DNAse I (Roche) at 4 ℃ for 30 min, and diluted to a total volume of 2.5 ml with IP2 buffer. IP with eIF3 antibodies was done as described for MCF7 cells, but beads were washed five consecutive times in the following buffers: Low-salt buffer (0.1% SDS, 1% Trition-X100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), high-salt buffer (0.1% SDS, 1% Trition-X100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 500 mM NaCl), LiCl buffer (250 mM LiCl,1% NP-40,1% deoxycholate,1mM EDTA,10mM Tris-HCl, pH 8.0), TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), and TE buffer again. After the final wash, beads we resuspended in 200 μl elution buffer (1% SDS, 1% $NaHCO_3$, 400U/ml RNase inhibitor).

Bound RNA was extracted with phenol:choloform:isoamyl alcohol followed by precipitation in ethanol precipitation at -20 ℃ overnight. cDNA were prepared by TransStart All-in-one First-Strand cDNA Synthesis SuperMix (TRAN, AT341) in a reaction containing 10 μl RNA , 10 μl RNAse-free water, 8 μl 5x TransStart mix and 2 μl gDNA Remover. Reactions were incubated at 42 °C for 15min, followed by inactivation at 85 °C for 5 s. Real time PCR samples were prepared by TransStart Tip Green qPCR SuperMix (TRAN, AQ141). The system contained 5 μl 2x TransStart Tip Green qPCR SuperMix, 0.2 μM of each primer, and 4.8 μl of the diluted cDNA. Reactions were processed for one cycle at 94 °C for 30 s, followed by 40 cycles at 94 °C for 5 s and at 60 °C for 30s in an ABI StepOne real time PCR machine. The comparative 2ΔCt method was used to assess relative enrichment of mRNAs in the eIF3-IP versus IgG control samples according to the formula: Fold enrichment = 0.5^ { [Ct(gene A in eIF3-IP)-Ct(gene A in input sample)] - [Ct(gene A in IgG-IP)-Ct(gene A in input sample)] }.

Supplementary Table 2 Oligonucleotides used

Primers for q-PCR	
Renilla luciferase forward	TAA CGC GGC CTC TTC TTA TTT
Renilla luciferase reverse	GAT TTG CCT GAT TTG CCC ATA C
beta-Actin forward	ATA GCA CAG CCT GGA TAG CAA CGT AC
beta-Actin reverse	CAC CTT CTA CAA TGA GCT GCG TGT G
ATP5L forward	GGT GAA CGC TGC TGT GAC TTA
ATP5L reverse	CAG CAC AGC TTC CTT AAC TGT G
ATP5C1 forward	GAG CTG AGA GAG AGC TGA AAC
ATP5C1 reverse	CGA TCT GAG GAC ACA CCA ATA A
ATP5O forward	GAC CTC TGC ATC TCC TTT AG
ATP5O reverse	TGA CGG ATC AGT CTT AGC CTC
ATP5α forward	GTA GGC GAG TTG GTC TGA AAG
ATP5α reverse	CCA AGC TAT CCA CAG CCT TAA T
ATP5B forward	CCC AGA ACA GGC CTT CTA TAT G
ATP5B reverse	TGC TCT TCA GCC AGC TTA TC
ATP5H forward	AGC TAT CGA CTG GGC TTA CTA
ATP5H reverse	CAT CCA CCT GGG CAG TAT ATT T
UQCRB forward	CCT AAA GAG CAG TGG ACC AAA TA
UQCRB reverse	GAC ACT CAA TGG GCT GAT CTT
UQCRC1 forward	GAG CAC CAG CAA CTG TTA GA
UQCRC1 reverse	TGA AGC GGC ATG GAG TAA G
UQCRC2 forward	CTC AGC AGC CAT TTG ATG TTT C
UQCRC2 reverse	TGT GGC CTG GGA GAT AGT ATA A
SDHB forward	CCT TAA TAA ATG TGG CCC CAT G
SDHB reverse	CAC AGA TGC CTT CTC TGC AT
GAPDH forward	CCC TTC ATT GAC CTC AAC TAC A
GAPDH reverse	ATG ACA AGC TTC CCG TTC TC
PSMB6 forward	GTT TCC ACT GGG ACC ACT ATC
PSMB6 reverse	GTC ACT CGA TTG GCG ATG TA
Primers for RT-PCR	
ght5 forward	ATT GGT GCT CTT TGG CAA TC
ght5 reverse	TTG AAC ACC ACG GGA TTT TT
erg2 forward	GTC GCC AGG AAA AAC GAT
erg2 reverse	CAA GGT AGC GAT TGA AGA TTC C

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