Supplemental Materials and Methods

Immunoblot studies
In brief, cells were lysed in NP-40 buffer (50 mM HEPES, pH 7.0, 150 mM NaCl, 2 mM EDTA, 25 mM NaF, 25mM β-glycerophosphate, 2 mM Na3VO4, 1% IGEPAL, and Complete Mini protease inhibitor cocktail tablet +/- EDTA; Roche) or RIPA lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris, pH 7.4, 2 mM Na3VO4, 25 mM β-glycerophosphate, 15 mM NaF, and complete protease inhibitor mix; Roche) and lysate cleared by centrifugation at 13,000 rpm for 10 min at 4°C. Lysate protein concentration was determined for each sample using BCA assay. Equal amounts of lysate resolved by 10 or 12% SDS-PAGE and transferred to a PVDF transfer membrane. Membrane was blocked in 5% BSA in TBS-T at 4°C. Primary antibodies were incubated at 4°C overnight. Secondary ECL antibodies (GE Healthcare) were incubated for 1 h at RT in 5% Milk in TBS-T. Protein was imaged using chemiluminescence method and Genemate autoradiography film. The following antibody dilutions were used: mouse anti-eIF4E (BD Bioscience #610270, 1:5000), rabbit anti-P-eIF4E S209 (Abcam #ab76256, 1:10000), rabbit anti-eEF2 (Cell Signaling #2332, 1:10000), rabbit anti-β-actin (Cell Signaling #4967, 1:10000), rabbit anti-4E-BP1 (Cell Signaling #9644, 1:10000), rabbit polyclonal anti-eIF4G1 (gift from N. Sonenberg, 1:1000), rabbit anti-eIF4A (Cell Signaling #2013, 1:5000), rabbit anti-P-eIF4E-BP1 S65 (Cell Signaling #9451, 1:1000), rabbit anti-S6 ribosomal protein (Cell Signaling #2217, 1:5000), rabbit anti-Akt (Cell Signaling #9272, 1:1000), rabbit anti-P-S6 ribosomal protein S235/236 (Cell Signaling #2211, 1:7000), rabbit anti-mTOR (Cell Signaling #2983, 1:1000), rabbit anti-TSC2 (Cell Signaling #3612, 1:1000), rabbit anti-eIF4B (Cell Signaling #3592, 1:1000), mouse anti-PABP (Abcam #ab6125, 1:1000), rabbit anti-eIF2α (Cell Signaling #9722, 1:1000), rabbit anti-eIF3H (Cell Signaling #3413, 1:1000), rabbit anti-eIF6 (Cell Signaling #3833, 1:1000), mouse anti-Runx2 (Millipore #05-1478, 1:1000), and rabbit anti-β-tubulin (Cell Signaling #2146, 1:1000). Quantitation was performed by determination of the integrated density of the bands using ImageJ software.

35S-Methionine metabolic protein labeling
Global protein synthesis was measured as previously described (Ramírez-Valle et al., 2008). Cells (2x10^5) were seeded, in triplicate, into 6-well culture plates and allowed to
Cells were labeled with 25 µCi $^{35}$S-methionine/cysteine protein labeling mix (PerkinElmer) for 30 min at 37°C using DMEM without L-Methionine or L-Cysteine supplemented with 5% FBS. Cells were lysed in NP-40 buffer (50 mM HEPES, pH 7.0, 150 mM NaCl, 2 mM EDTA, 25 mM NaF, 25 mM β-glycerophosphate, 2 mM Na$_3$VO$_4$, 0.5% IGEPAL, and complete protease inhibitor tablet; Thermo Scientific). Lysates were cleared by centrifugation at maximum speed for 15 min at 4°C. Proteins were precipitated in 10% TCA followed by scintillation counting. Protein concentration was measured by the BCA assay (Thermo Scientific). For studies involving doxycycline treatment, Dox was added 48 h after plating and maintained throughout the course of the study.

**pTripz construct expression**

The tetracycline responsive element (TRE) was induced using 1-2 µg/ml of doxycycline was administered to cells for 48-96 h. When possible, RFP expression was analyzed through fluorescent microscopy or FACS analysis.

**Generation of Inducible Constructs**

shRNA cassettes were cloned into 5’-Xhol and 3’-EcoRI sites of Inducible TRIPZ vector (pTripz). Constructs were transformed into One Shot Stbl3 cells (Invitrogen) and grown on LB-amp/zeo plates overnight at 37°C. Plasmids were purified from individual colonies and sequenced. Sequencing primer: (5’-GGAAAGAATCAAGGAGG-3’). Overexpression constructs were generated following a similar method except the ORFs were cloned into 5’-AgeI and 3’-MluI sites and sequenced with the following primers: Fwd: (5’-CGAGGTTCTAGACGAGTTTA-3’); Rev: (5’-GCCTTAAGAACCCCAGTATCAG-3’). The shRNA cassette sequences are as follows: eIF4E-1 (5’-CACAATAGTCAGAAAACAACT-3’), eIF4E-2 (5’-GCGTCAAGCAATCGAGATTTG-3’), TSC2 (5’-CAGCATTAATCTCTTACCAT-3’) and Runx2 (5’-CCACAGAATTTGCATTTAGAG-3’). The eIF4E and 4E-BP1 ORFs were subcloned from the pBABE-puro retrovirus vector using the AgeI/MluI cloning sites. Point mutations were made using site-directed mutagenesis (Agilent Technologies).

**Generation of stable cell lines**

HEK293FT cells were transfected with pTripz lentivirus vector, pS Pax2, and PMD2.G using lipofectamine 2000 (Life Technology) for 6-8 h. Media was changed to DMEM
(Corning) supplemented with 5% heat-inactivated FBS (Gibco). Virus was collected 48 and 72 h after transfection. Virus was concentrated and either used immediately to transduce MCF7 cells or stored at -80°C. Cells were infected in the presence of Polybrene (5-10 µg/ml) and thereafter selected for resistance to puromycin (Sigma) or geneticin (Gibco) for up to one week.

**Immunohistochemistry (IHC) and scoring**

All kits and developing substrates were obtained from Vector Laboratories. Paraffin-embedded tumor sections (4 µm thickness) were de-paraffinized in xylene and ethanol, rehydrated and subjected to antigen retrieval by microwaving for 30 min in antigen unmasking solution (Vector Laboratories), then 5% H₂O₂ to block endogenous peroxidase activity for 30 min at RT followed by protein blocking of non-specific epitopes with 1.5% normal horse serum (Vector Laboratories). Slides were incubated with primary antibodies for 4E-BP1 and P-4E-BP1 (S65, Cell Signaling), P-eIF4E (S209, Abcam), S6 and P-S6 (S240/244, Cell Signaling), and eIF4E (BD Transduction) overnight at 4°C. After washing with PBS-T, slides were incubated with secondary antibody for 2 h at RT and DAB staining carried out according to manufacturer instructions (Vectastain ABC kit, Vector Laboratories). Slides were counterstained with hematoxylin. Specimens were analyzed by a pathologist blinded to the study and 20% of specimens chosen at random spot scored by a second pathologist also blinded to the study. Specimens were scored as 0, 1, 2, 3+ with a cut-off for staining that had to include at least 5% of the cells in a given population and took into consideration the percentage of positive cells as follows. For quartile scoring: 0 <5%; +1 ≥6% but <33%; +2 >33% but <65%; +3 >66%. For tertile staining: 0 <5%; +1 ≥6% but <50%; +2 ≥50%.