SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Spheroids and xenografts contain hypoxic cores with inactive mTORC1. (A) Immunolabeling of a four day old spheroid section of U87MG glioblastoma cancer cells incubated with Hypoxyprobe™ for 1 h. (B) Immunolabeling of a four day old spheroid section of glioblastoma cancer cells with anti-phosphorylated 4E-Binding Protein (P4E-BP) shows that mTORC1 is inactive in the core. (C) Immunolabeling of a four day old spheroid section of glioblastoma cancer cells with anti-4E-Binding Protein (4E-BP). (D) Sections of an excised four week old nude mouse xenograft of glioblastoma cancer cells taken from the edge and the core. Immunolabeling with anti-P4E-BP shows that mTORC1 is active at the surface and inactive in pockets in the core. (E) Hematoxylin and eosin (H&E) stain of a four week old nude mouse xenograft core section of glioblastoma cancer cells. Arrows, blood vessels. Hoechst was used as a DNA counterstain. Solid line, section border. Dotted line, border between edge and core. Scale bar, 100 µm.

Figure S2. Cancer cells require eIF4E2-directed protein synthesis to survive and proliferate under hypoxia. (A) Two shRNAs were used independently to silence eIF4E2. One shRNA (shRNA-1) targeted the eIF4E2 Coding Sequence (CDS) and the other (shRNA-2) targeted the 3’UTR. Three clones displaying eIF4E2 knockdown were selected from three genetically diverse cancer cell lines (U87MG glioblastoma, 786-O renal cell carcinoma and HCT116 colorectal carcinoma) stably expressing shRNA targeting the eIF4E2 CDS (1.1, 1.2 and 1.3) or the eIF4E2 3’UTR (2.1, 2.2 and 2.3). Multiple clones were also generated in all three cell lines that stably express a non-targeting control shRNA (C1.1, C1.2 and C1.3). See also Fig. 2A. (B) Western blot analyses were performed to determine the ability of each clone to induce EGFR when exposed to 24 h of normoxia (21% O2) or hypoxia (1% O2). GAPDH was used as a loading
control. (C) The ability of U87MG glioblastoma, 786-O renal cell carcinoma and HCT116 colorectal carcinoma cells stably expressing control shRNA (C1.2 and C1.3) or shRNA targeting eIF4E2 (1.2, 1.3, 2.2 and 2.3) to proliferate in culture was measured by BrdU incorporation following incubation in normoxia or hypoxia for 24 h. (D) Cell number was reported for glioblastoma, renal cell carcinoma and colorectal carcinoma eIF4E2-depleted cells (1.1, 1.2, 1.3, 2.1, 2.2, and 2.3) exposed to 48 h of normoxia or hypoxia relative to control cells (C1.1). Columns, mean (n = 3); error bars, s.e.m. Significance measured by student’s t test * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure S3. Cancer cells require eIF4E2-directed protein synthesis to evade cell death. (A-C) The ability of U87MG glioblastoma (A), 786-O renal cell carcinoma (B) and HCT116 colorectal carcinoma (C) cells stably expressing control shRNA (C1.1) or shRNA targeting eIF4E2 (1.1 and 2.1) to evade apoptosis in culture was measured by flow cytometry following incubation in normoxia or hypoxia for 48 h. The apoptotic population is indicated by the black line (sub-G1). Counts. cell number. (D) The ability of glioblastoma, renal and colorectal cancer cells to form colonies after exposure to normoxia or hypoxia was measured in cells stably expressing control shRNA (C1.1) or shRNA targeting eIF4E2 (1.1, 1.2, 2.1, and 2.2). A representative image of colorectal carcinoma colonies stained with crystal violet is shown.

Figure S4. Normal cells require eIF4E2-directed protein synthesis to survive and proliferate under hypoxia. (A) The ability of human renal proximal tubular epithelial cells (HRPTEC) transiently expressing control shRNA (Ctrl) or shRNA-1 or -2 targeting eIF4E2 (KD1 and KD2) to proliferate in culture was measured by BrdU incorporation following incubation in normoxia or hypoxia for 24 h. BrdU-positive cells were reported relative to cells stably expressing non-targeting shRNA. (B) % cell death was measured for eIF4E2-depleted or control HRPTEC
exposed to 48 h of normoxia or hypoxia by dividing trypan blue-positive cells by the total number of cells. (C) Western blot analysis of eIF4E2 protein levels in HRPTEC transiently expressing shRNA-1 (KD1) or shRNA-2 (KD2) targeting eIF4E2, or non-targeting control shRNA (Ctrl). HIF-2α was used as a marker of hypoxia. GAPDH was used as a loading control. Columns, mean (n = 3); error bars, s.e.m. Significance measured by student’s t test *** p < 0.001.

**Figure S5.** eIF4E2 loss prevents tumor growth and reduces the growth of already-established tumors. (A) Mice were injected with 10⁶ U87MG glioblastoma cells stably expressing non-targeting shRNA (C1.1 and C1.2) or shRNA targeting eIF4E2 (1.1, 1.2, 2.1 and 2.2). A representative mouse was photographed at the ethical endpoint. (B-C) The U87MG glioblastoma (B) and HCT116 colorectal carcinoma (C) xenografts were confirmed to be infected with lentivirus by detecting the GFP (green) being expressed by the pGIPZ backbone. Western blot analysis of eIF4E2 protein levels in lentivirus-infected established glioblastoma and colorectal carcinoma xenografts. GAPDH was used as a loading control. Ctrl, control. KD, eIF4E2 knockdown. Hoechst was used as a DNA counterstain.

**Figure S6.** Spheroid growth requires eIF4E2-directed protein synthesis. (A) Light micrograph of a U87MG glioblastoma control spheroid (C1.2) compared to an eIF4E2-depleted spheroid (2.1). Scale bar, 100 μm. (B) Spheroid growth was monitored over a period of four days in U87MG glioblastoma, 786-O renal cell carcinoma and HCT116 colorectal carcinoma cells stably expressing control shRNA (C1.2 and C1.3) or shRNA targeting eIF4E2 (1.2, 1.3, 2.2 and 2.3). Data are mean ± s.e.m. of three independent experiments. Significance measured by student’s t test * p < 0.05, ** p < 0.01, *** p < 0.001. (C) Western blot analyses of four day old spheroids from glioblastoma and colorectal carcinoma cells stably expressing control shRNA or shRNA
targeting eIF4E2. HIF-2α was used as a marker for hypoxia and hypoxic protein synthesis. GAPDH was used as a loading control.

**Figure S7.** Reintroduction of exogenous eIF4E2 restores tumor cell proliferation. (A) Western blot analysis were performed to determine the ability of each rescue (2.3Y and 2.3Z) to induce EGFR when exposed to 24 h of normoxia (21% O₂) or hypoxia (1% O₂). GAPDH was used as a loading control. (B) Cell number was reported for vehicle control eIF4E2 knockdown cells (2.1v1, 2.1v2, 2.3v1 and 2.3v2) exposed to 48 h of normoxia or hypoxia relative to eIF4E2 rescues (2.1Y, 2.1Z, 2.3Y or 2.3Z). (C) The ability of vehicle control eIF4E2 knockdown cells (2.1v2 and 2.3v2) to proliferate in culture relative to eIF4E2 rescues (2.1Z and 2.3Z) was measured by BrdU incorporation following incubation in normoxia or hypoxia for 24 h. (D) Active caspase-3-positive cultured vehicle control eIF4E2 knockdown cells (2.1v2 and 2.3v2) reported relative to eIF4E2 rescues (2.1Z and 2.3Z) incubated in normoxia or hypoxia for 48 h. (E) Spheroid growth was monitored over a period of four days in vehicle control eIF4E2 knockdown cells (2.1v2 and 2.3v2) and eIF4E2 rescues (2.1Z and 2.3Z). Data are mean ± s.e.m. of three independent experiments. (F) Western blot analysis of a four day old spheroid from vehicle control eIF4E2 knockdown cells (2.1v1) and eIF4E2 rescues (2.1Y). HIF-2α was used as a marker for hypoxia and hypoxic protein synthesis. GAPDH was used as a loading control. (G) Weekly tumor volume measurements in nude mouse xenograft assays performed with vehicle control cells (2.1v2 and 2.3v2) and eIF4E2 rescues (2.1Z and 2.3Z). Representative mice that were injected subcutaneously with eIF4E2 rescues on the left flank and vehicle control cells on the right flank were photographed at the ethical endpoint. Data are mean ± s.e.m. of at least three independent experiments. (B-D) Columns, mean (n = 3); error bars, s.e.m. Significance measured
by student’s t test * p < 0.05, ** p < 0.01, *** p < 0.001. All experiments performed in U87MG glioblastoma.