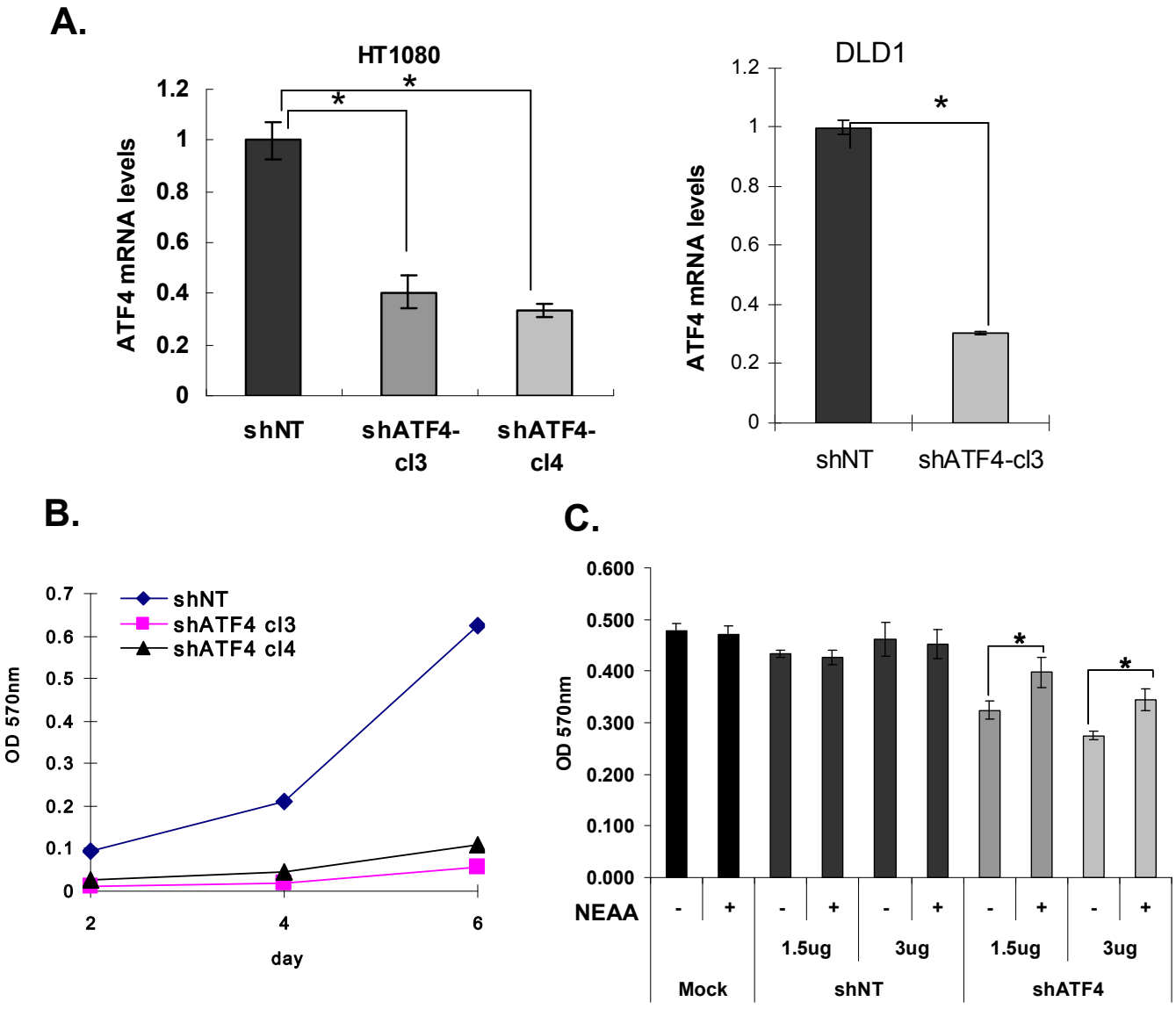
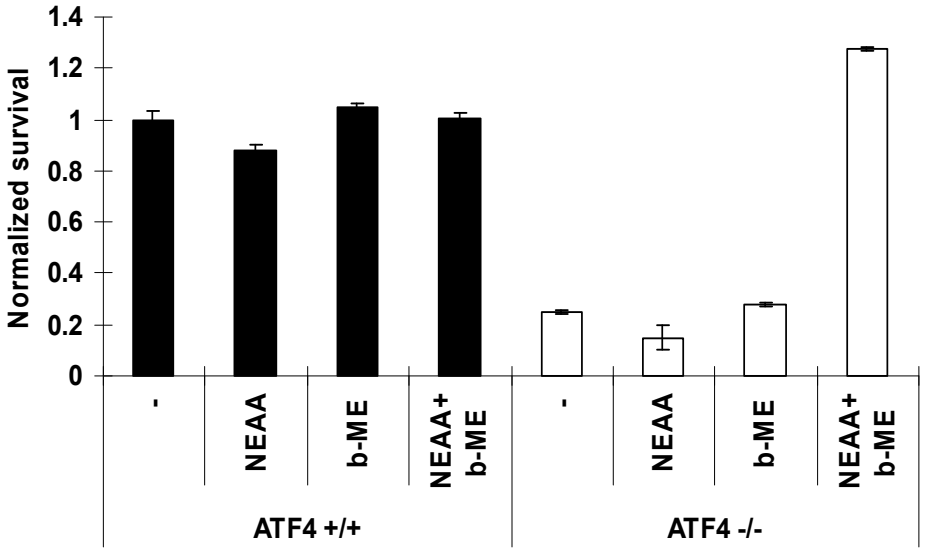
Figure S1



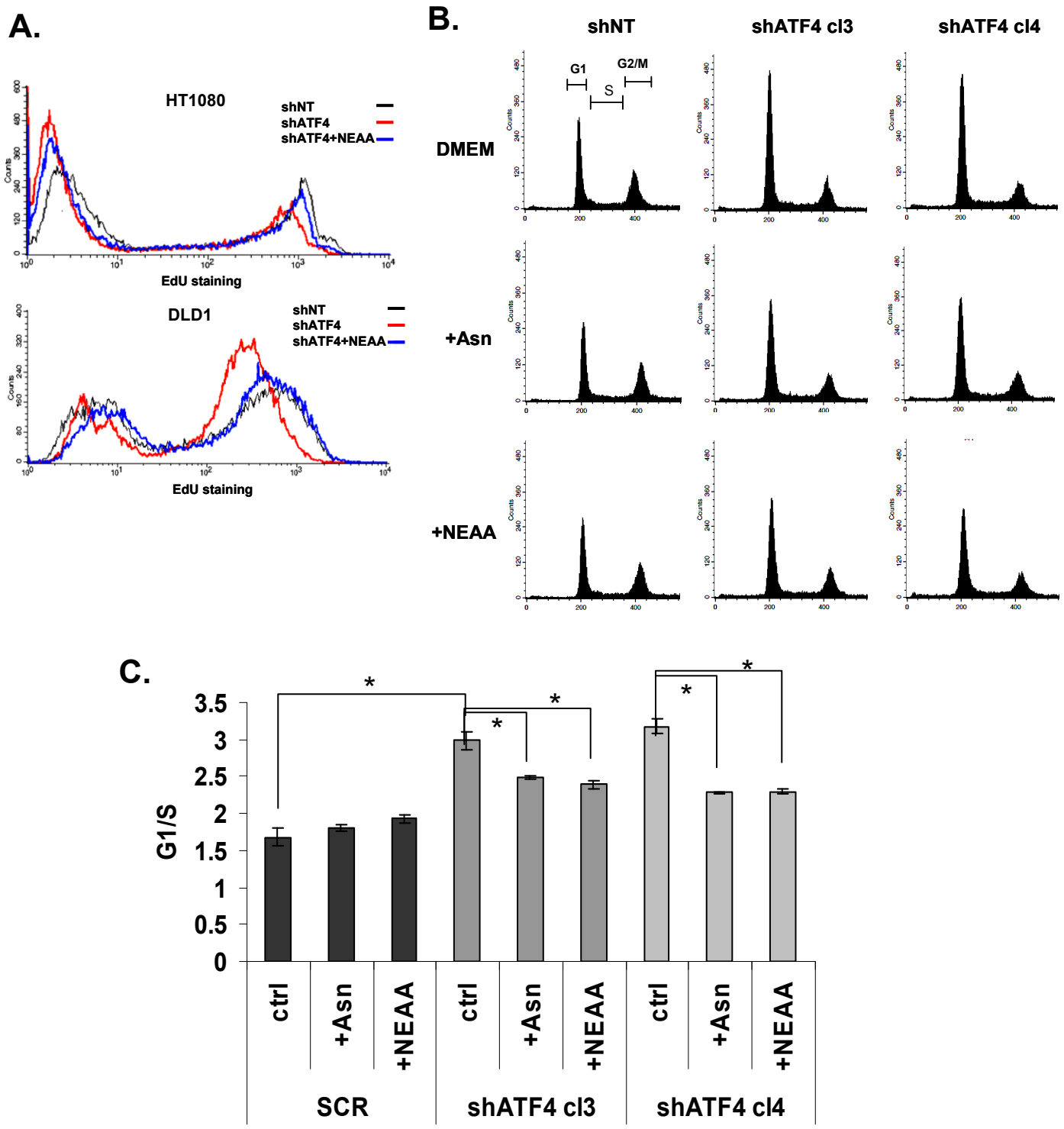
Sup figure 1: (A) Real time RT-PCR analysis for ATF4 mRNA levels in HT1080 and DLD-1 clones expressing none-targeting shRNA (shNT) or shRNA targeting human ATF4 (shATF4). mRNA levels were normalized against 18s rRNA internal control and are reported as a fraction compared to levels in shNT control cells. PCR reactions were done in triplicate. Error bar represents standard error. * $p<0.05$. Two-tailed Student's t-test. (B) HT1080 cells were incubated in DMEM for 2,4 and 6 days. Cell growth was measured using MTT assay. (C) HT1080 cells transiently transfected with shNT or shATF4 shRNA were incubated in DMEM +/- NEAA for two days, cell survival were measured using MTT assay.

Figure S2



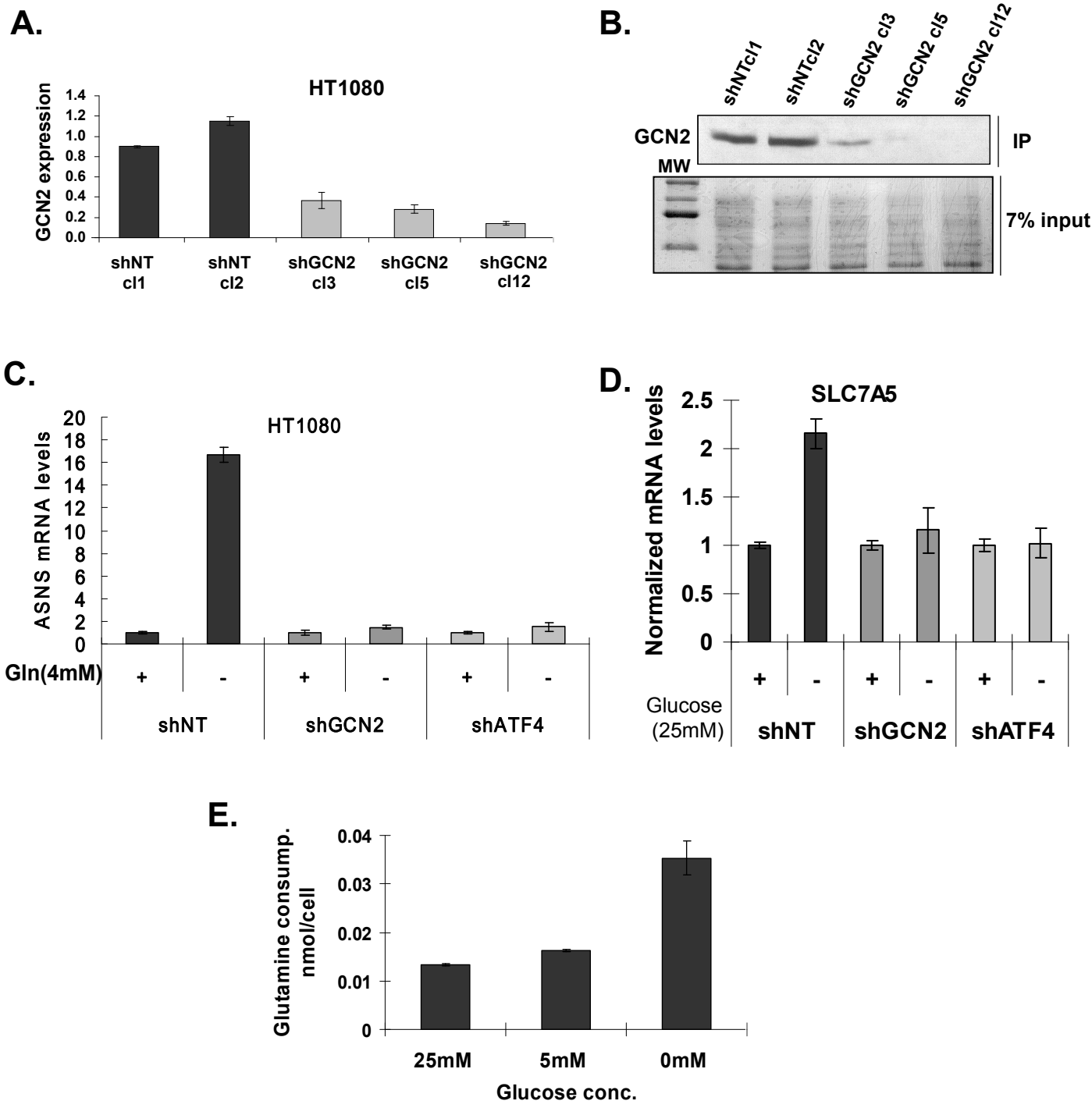
Sup figure 2: ATF4$^{+/+}$ and ATF4$^{-/-}$ MEFs were incubated in DMEM with supplements indicated for 48h. Cell survival was measured using MTT assay.

Figure S3



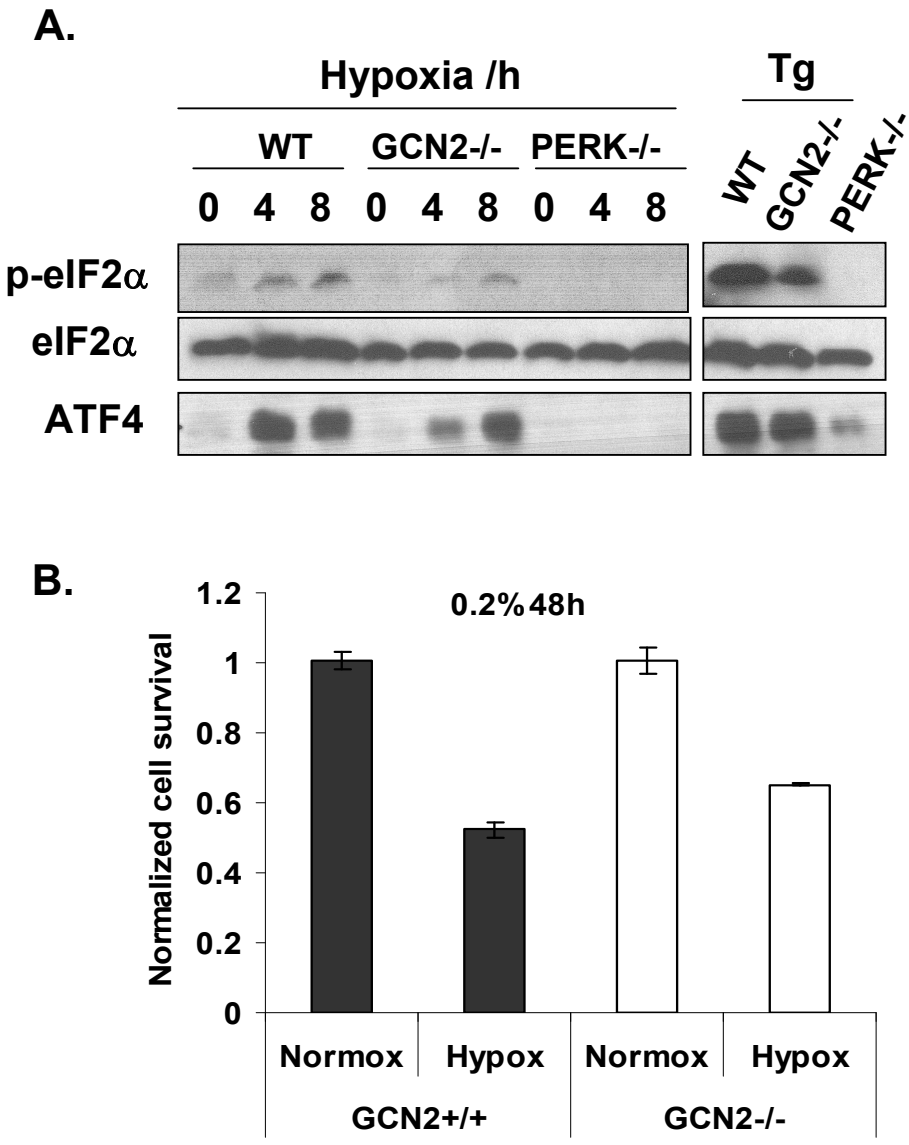
Sup figure 3: (A) Cell proliferation of HT1080 and DLD1 cells measured using flow cytometry EdU assay. EdU-: EdU negative cells, arrested population; EdU+: EdU positive cells, proliferating population. Cells were incubated in DMEM with amino acids for 24h. **(B)** Cell cycle was analyzed by propidium iodide (PI) staining using CellQuest (Becton Dickinson). Cells were incubated in DMEM with amino acids for 24h. **(C)** Quantitation of G_1/S ratio from 3 independent experiments using the indicated gates in (B). Error bars represent S.E., N=3. *p<0.05, student's t test.

Figure S4



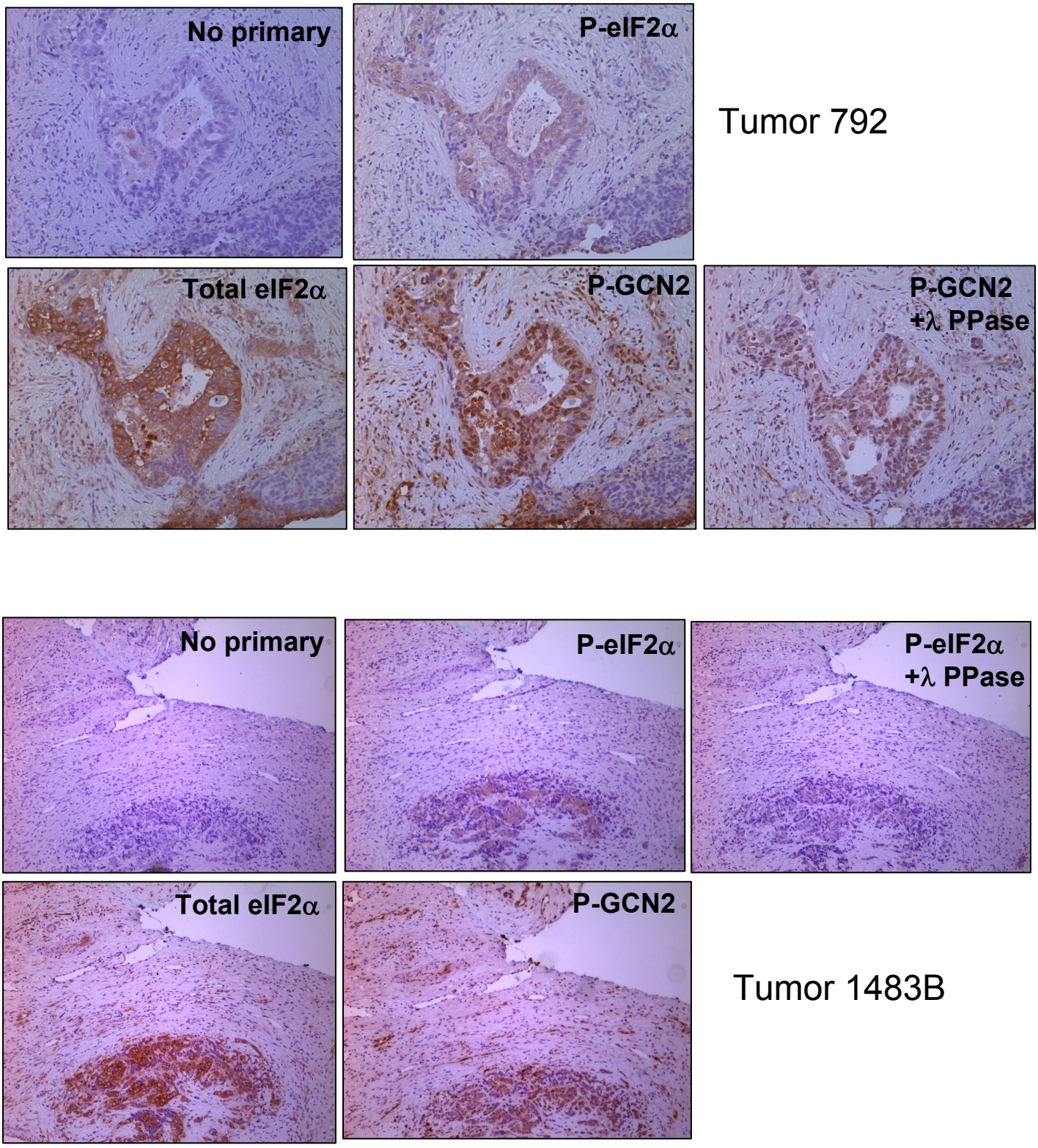
Sup figure 4: **(A)** Real time qPCR analysis for GCN2 mRNA levels in HT1080 clones expressing non-targeting shRNA (shNT) or shRNA targeting human GCN2 (shGCN2). mRNA levels were normalized against 18s rRNA levels and are reported as a fraction compared to levels in shNT1 control. PCR reactions were done in triplicate. Error bars represent S.E. * $p<0.05$, Two-tailed Student's t-test. **(B)** GCN2 knockdown assayed by immunoprecipitation. Ponceau staining for 7% of input cell lysate (bottom) was used as a loading control. **(C**) HT1080 cells were incubated in full DMEM or DMEM (-Gln) for 8h. Total RNA were extracted for real-time PCR. **(D)** HT1080 cells were incubated in DMEM with/without 25mM glucose for 8h. Total RNA was extracted for real-time PCR. **(E)** HT1080 cells were cultured in DMEM with 0, 5 and 25mM glucose. Glutamine consumption was measured 16h later and normalized to cell number.

Figure S5



Sup figure 5: **(A)** Wild type, GCN2-/- and PERK-/- MEFs were exposed to 0.2% oxygen for up to 8h, cells were harvested for immunoblot. 0.5μM thapsigargin treatment for 3h was used as a positive control. **(B)** GCN2+/+ and GCN2-/- MEFs were exposured to 0.2% oxygen for 48h. Cell survival was analyzed using MTT assay. (Data represent mean ± SEM, n = 3, *p < 0.05.)

Figure S6



Sup figure 6: Immunohistochemical analysis of P-eIF2α, P-GCN2, total eIF2α and total GCN2 in colorectal cancer metastases to the liver from two human patients. Tissues were processed as described in materials and methods and incubated with the indicated primary antibodies (or no primary Ab as controls). Moreover, to verify the specificity of the phospho-specific anti-eIF2a and anti-GCN2 antibodies, separate serial sections were treated with λ phosphatase prior to primary antibody incubation. Tumor 1483B depicts a different area from the same tumor shown in Fig. 9D

Table S I

	Conc. of NEAA added/μM	Conc. In DMEM/μM
Alanine	100	0
Aspartic acid	100	0
Asparagine	100	0
Glutamic acid	100	0
Glycine	100	400
Proline	100	0
Serine	100	400

SUPPLEMENTARY METHODS

Immunofluorescence

Fresh-frozen tumors were sectioned (10μm) and fixed to glass slides in 4% formaldehyde for 30 min. Sections were then blocked in 3% BSA in PBS for one hour. For detection of proliferation a Ki-67 antibody (sc-15402 , Santa Cruz Biotechnology Inc.) was used at dilution 1:50. followed by Cy3-conjugated secondary (ZyMax Goat Anti-Rabbit IgG from Invitrogen). Fluorescence immunostaining was detected using Nikon eclipse TE2000-U microscope and photographed at several magnifications. Experiments were performed in triplicate. Images of three different areas of each tumor section were captured and the quantitation of proliferating cells was determined using Matlab software.

Immunoblotting

Immunoblotting was performed as described previously (Koumenis et al., 2002). The antibodies used were: anti-ATF4 (Santa Cruz Biotechnology), anti-β-actin and anti-a-tubulin (Sigma-Aldrich), anti-p-eIF2a, anti-eIF2a, anti-p-GCN2, anti-GCN2, anti-cleaved caspase-3, anti-cleaved PARP, anti-LC3II (Cell Signaling Technology Inc.), anti-p21 (BD Pharmingen™), anti-p27 (BD Transduction Laboratories™), anti-ASNS (AbCam).

Tumor samples and Immunohistochemistry for GCN2

The mouse breast tumors arose from MMTV-Neu transgenic mice. Unaffected normal mammary glands were simultaneously harvested from the same animal. Tissues were immediately frozen on dry ice and stored at -80^{o}C. All the tissues were homogenized in lysis buffer using a ULTRA-TURRAX homogenizer (Janke & Kunkel).

Snap frozen, human tumor and normal tissues (liver, breast and lung) were obtained from the Tumor Tissue and Biospecimen Bank (TTAB) facility at the University of Pennsylvania, School of Medicine. Collection and processing of human specimens was performed in accordance to regulations of the Abramson Cancer Center and the Department of Pathology and Laboratory Medicine at the University Of Pennsylvania School of Medicine.

Multitumor tissue microarray slides (T-MTA-6A) were obtained trough the Tissue Array Research Program (TARP, NCI-Frederick). Formalin-fixed, paraffin-embedded tissue slides were deparafinized and rehydrated in distilled water followed by antigen retrieval using 10 mM sodium citrate (buffer pH 6.0) and endogenous peroxidase inactivation with 3% hydrogen peroxide. Sections were blocked with 5% normal goat serum and incubated in Rabbit polyclonal anti-Phospho-GCN2 antibody (1:50, Cell Signaling Technologies #3301), anti-Phospho-eIF2α antibody (1:25, Cell Signaling), or anti-eIF2α (1:50, Cell Signaling Technologies #9722) and goat anti-rabbit Horseradish Peroxidase secondary antibody (1:200, Jackson Laboratories).

As a control and to verify the phospho-specificity, separate serial sections were treated with lambda phosphatase (New England Biolabs #P0753) for 45 minutes at 37^{o}C prior to primary antibody incubation. For antigen detection we utilized the DAB chromogen method. Slides were counterstained in Mayer's hematoxylin, mounted and photographed using a Nikon microscope.

All the immunohistochemistry figures were photographed under identical conditions (e.g., exposure time, contrast, etc). Whenever contrast or brightness had to be adjusted, this was performed on the whole figure composite and not on individual panels.

Real-Time PCR Primer sequences

ATF4:
5’-TCCCATCTCCAGGTGTTCTC-3’ (forward),
5’-CAGCTCTTTGCACTCACCAG-3’ (reverse).

ASNS:
5'-TACAACCACAAGGCGCTACA-3' (forward),
5'-AAGGGCCTGACTCCATAGGT-3' (reverse).

SLC1A4:
5’-AGCTCAAC GCAGGACAGATT-3’(forward),
5’-ATTCAGGTGGTGGAGAATGC-3’(reverse).

SLC7A5:
5’-GGAGGCTGCTGTGAAAACTC-3’ (forward),
5’-AGGAGAAAGGA AGGCTCCTG-3’ (reverse).

GCN2:
5’-TGCCAACTTACATCAGAAAAGC (forward),
5’-TTTGAGGTATATTTGCTTTGG (reverse).