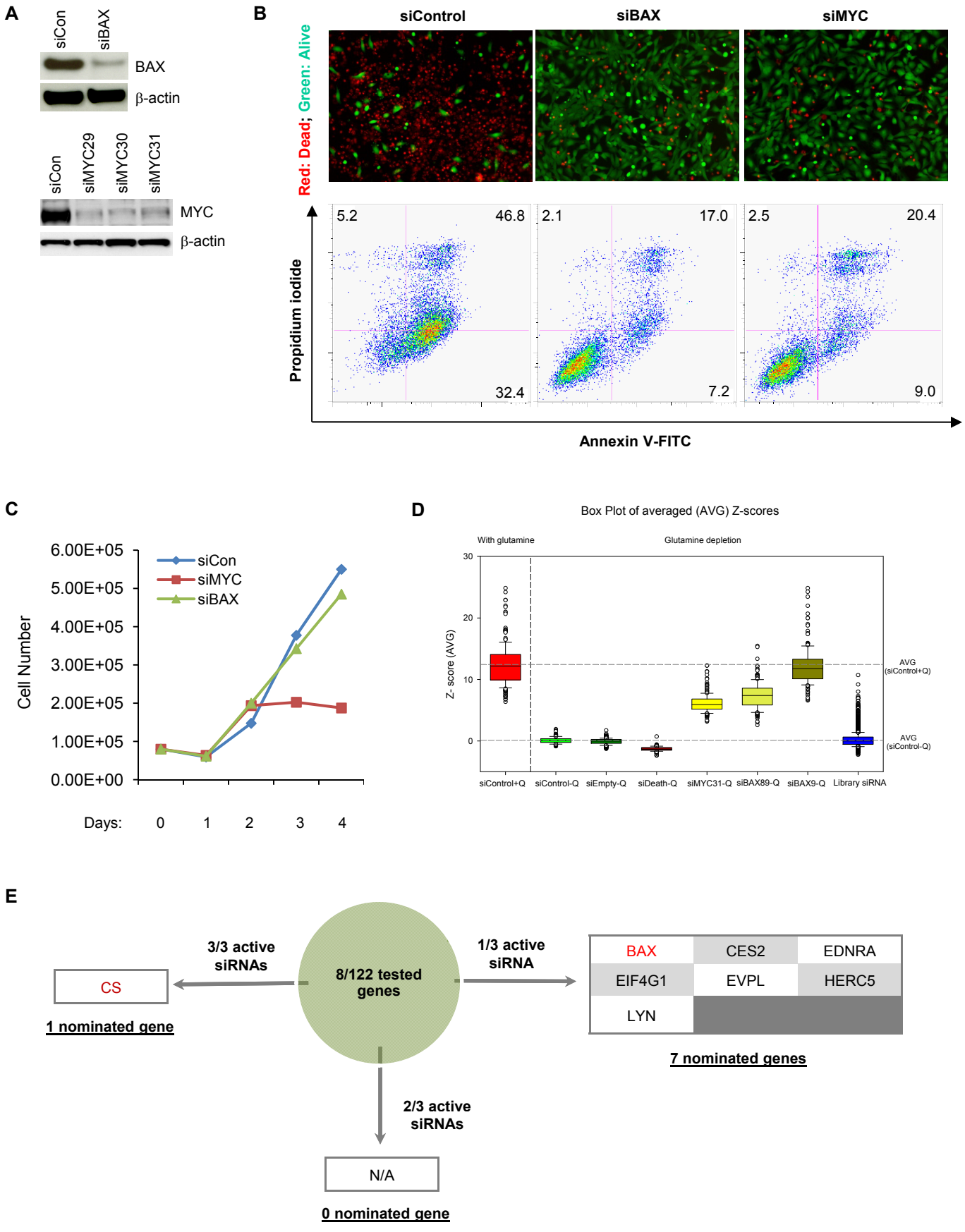
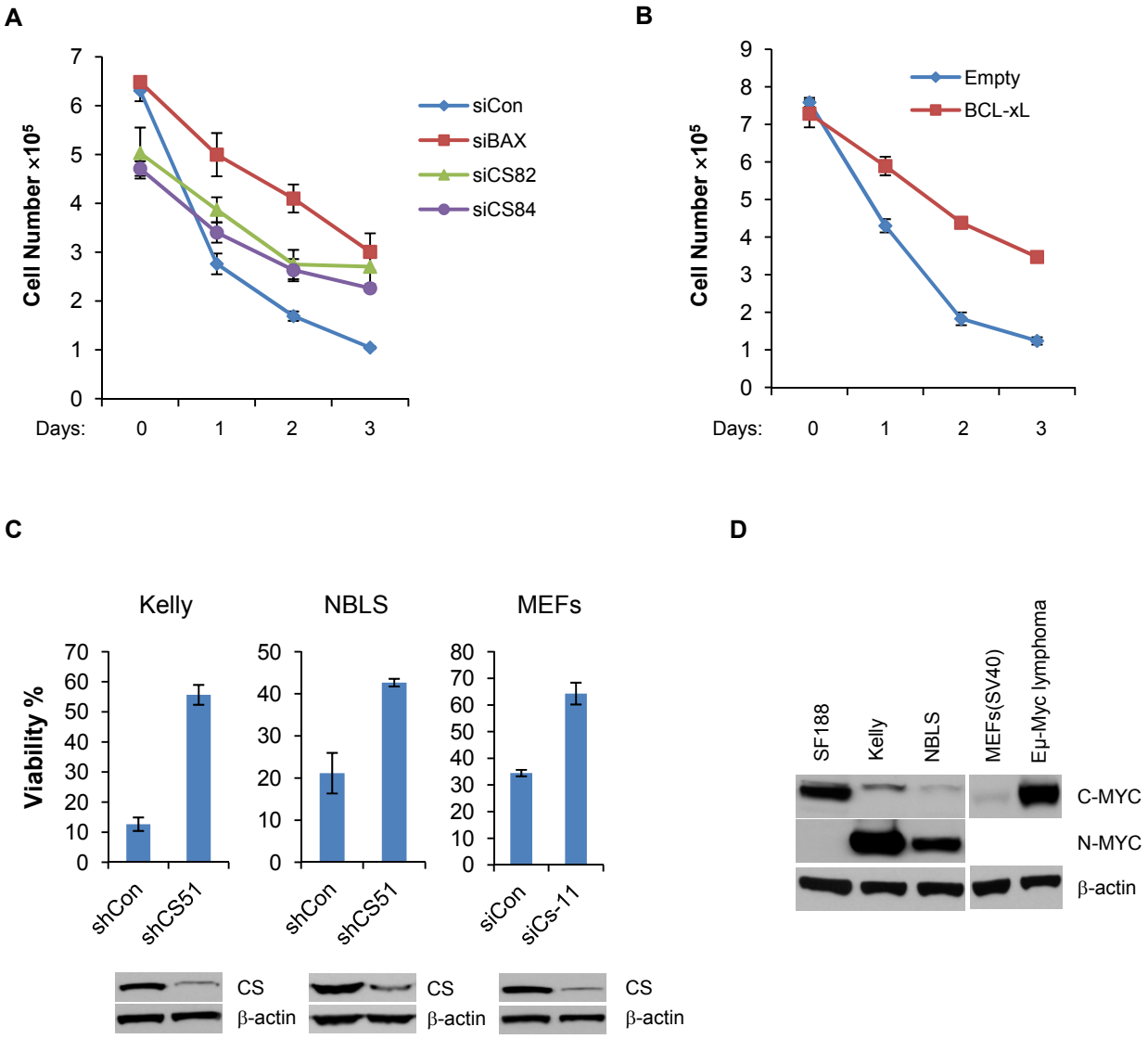
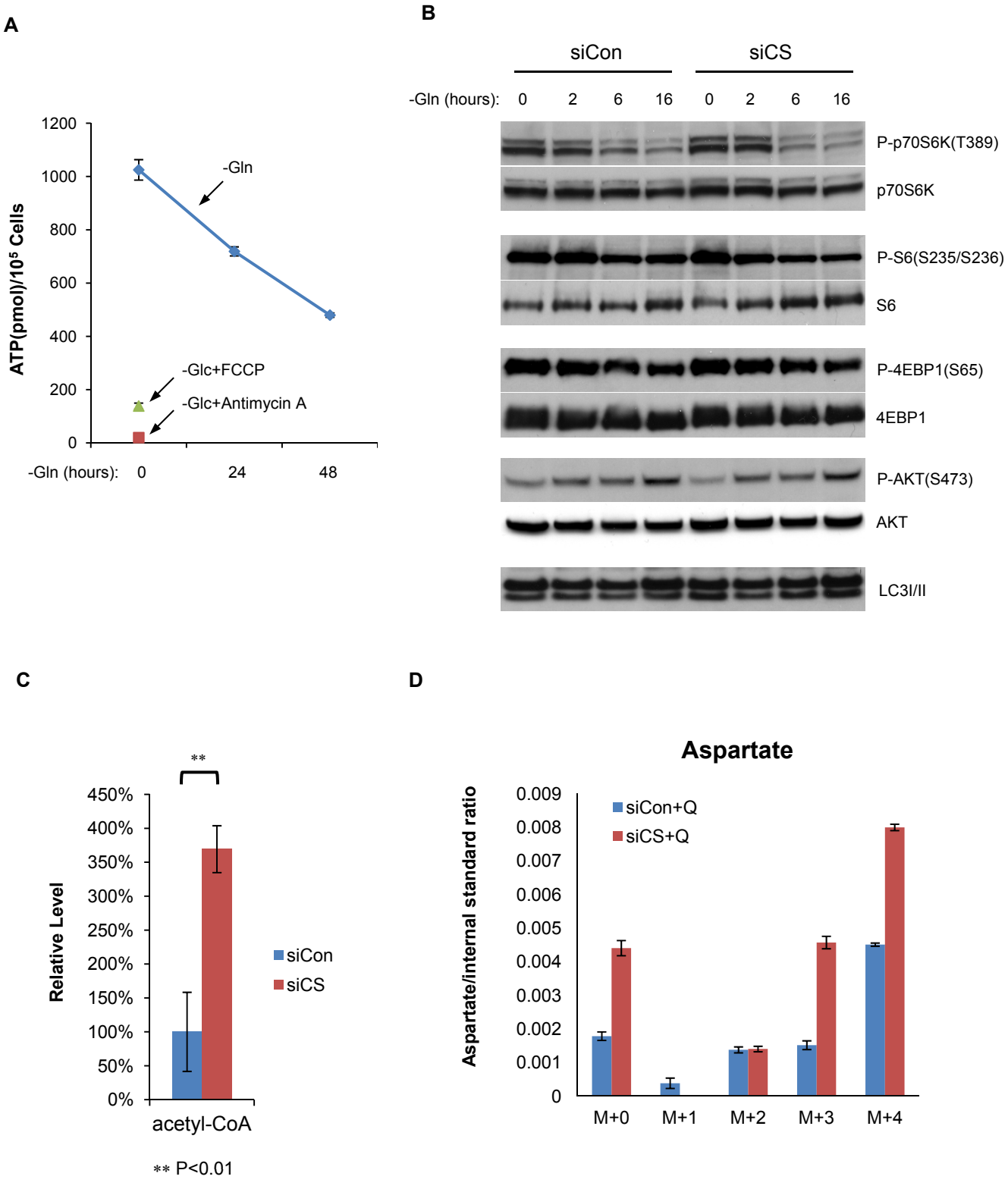
## Supplemental Figure 1



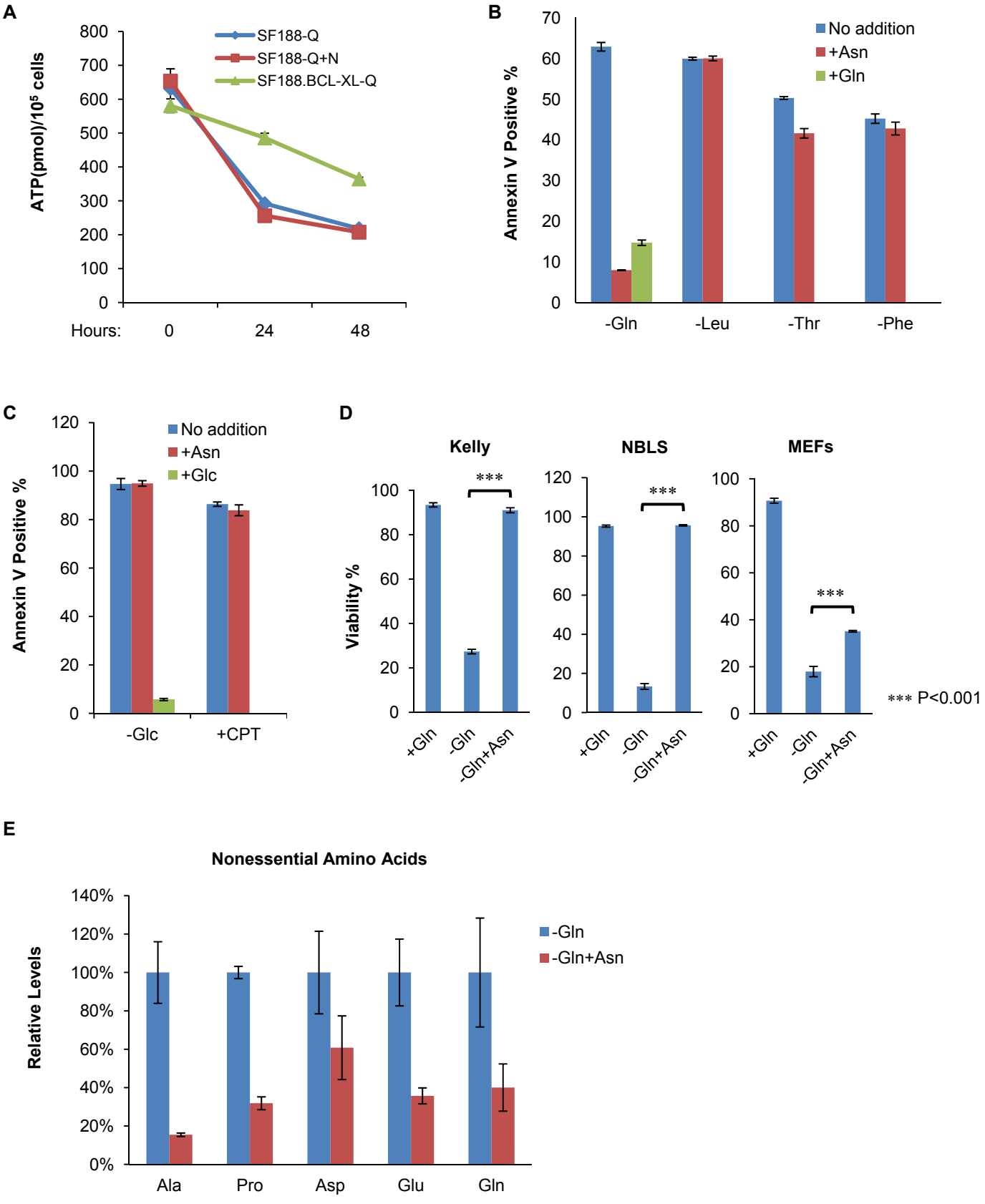
## Supplemental Figure 2



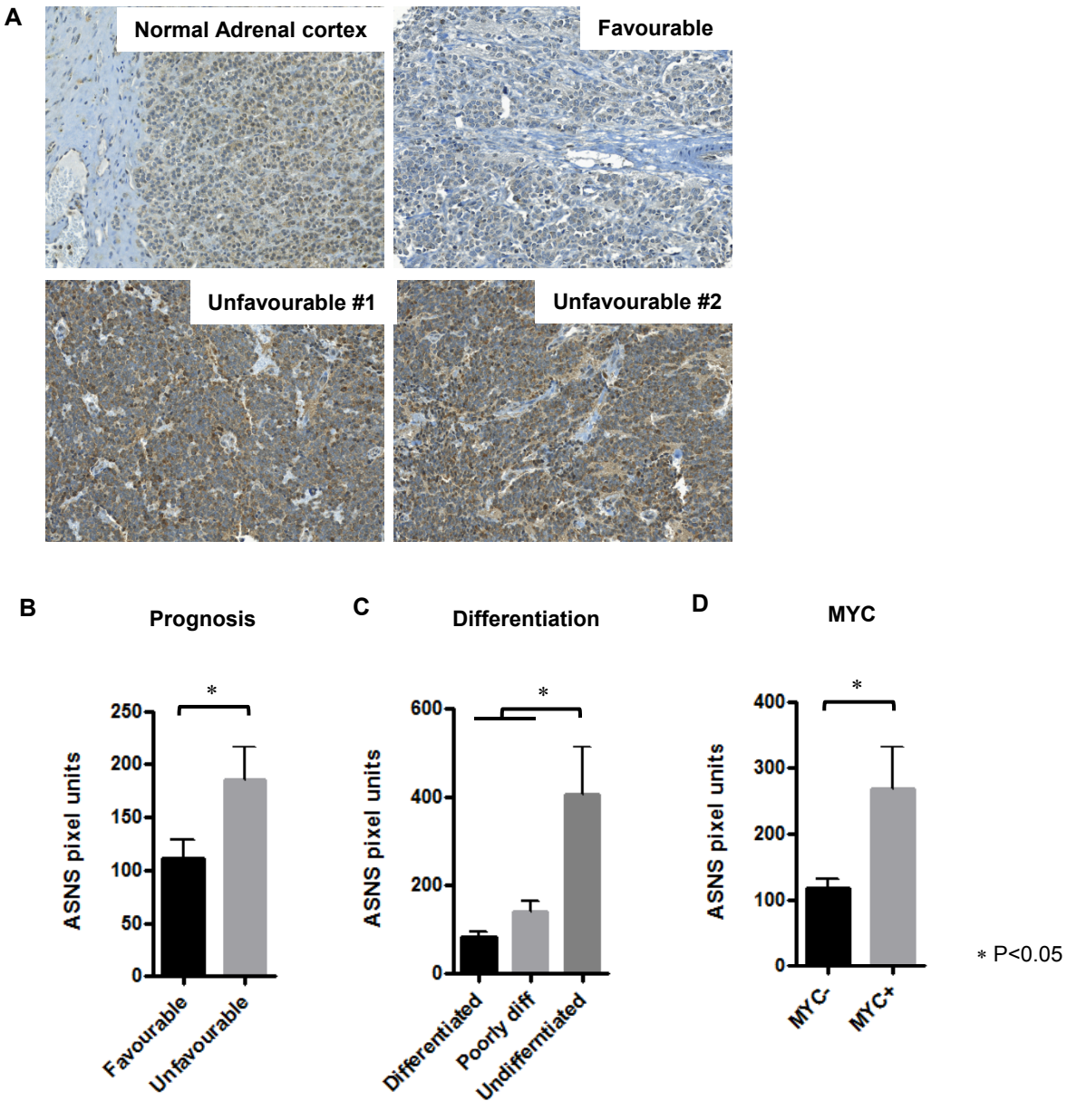
## Supplemental Figure 3



## Supplemental Figure 4



## Supplemental Figure 5



**Supplemental Figure 1, related to Figure 2. High throughput screen identifies citrate synthase siRNA as a suppressor of glutamine-depletion-induced apoptosis.**

(A) Western blotting of SF188 cells 2 days after transfection with siRNA for control or BAX and MYC shows reduction of protein levels.

(B) SF188 cells were transfected with siRNA against MYC and BAX for 48 hours. Glutamine was deprived for another 2 days. Cells were stained with calcein AM and ethidium homodimer-1 (L-3224, Life Technologies) to visualize live and dead cells respectively. Alternatively, cells were stained with Annexin V and PI, followed by flow cytometry to quantify apoptosis.

(C) SF188 cells were transfected with siRNA against MYC and BAX. Cell number was recorded for 4 days post-transfection in complete DMEM with glutamine.

(D) Average robust z-scores of the control siRNA and the same siRNA pairs in the library from the primary screen.

(E) A schematic representation of the nomination of hits in the secondary screen. CS was the only gene that had 3 siRNAs score over the threshold.

**Supplemental Figure 2, related to Figure 3. Knockdown of citrate synthase prevents cell death in multiple cell types.**

(A) SF188 cells were transfected with siRNA for BAX, CS or control for 48 hours. Glutamine was then withdrawn from the medium and the numbers of viable cells were recorded for 3 days post medium change.

(B) SF188 cells and SF188 cells with constitutively overexpressed BCL-$X_L$ were deprived of glutamine for 3 days. Total number of viable cells was recorded.

(C) Kelly and NBLS neuroblastoma cell lines were infected with lentivirus carrying shRNA for *hCS* or control and selected in the presence of puromycin (2 μg/mL) for 24 hours. The selected Kelly and NBLS cells were subjected to glutamine starvation for 4 days or western blotting to confirm efficient knockdown. SV40 transformed MEFs were transfected with siRNA against *mCS* by Nucleo4D (Lonza AG) for 2 days, and then subjected to glutamine starvation for 4 days or western blotting to confirm efficient knockdown.

(D) Whole cell extract was prepared from Kelly, NBLS and MEFs to confirm the expression of c-MYC or N-MYC. Extract from primary Eμ-Myc lymphoma was used as a positive control.

The data in Figure S2 (A, B and C) are shown as mean +/- S.D., n=3.

**Supplemental Figure 3, related to Figure 4. Knockdown of CS alters intracellular acetyl-CoA, but not mTOR signaling or autophagy.**

(A) SF188 cells with constitutively overexpressed BCL-$X_L$ were deprived of glutamine for 48 hours. Cellular ATP levels were recorded at 0, 24 and 48 hours and normalized to the number of viable cells. As a control for the assay, cells were treated with antimycin A (25 μg/mL) or FCCP (10 μM) in the absence of glucose (-Glc) for 30 minutes prior to the assay.

(B) SF188 cells were transfected with control or CS siRNA for 2 days and then subjected to glutamine withdrawal for 16 hours. Protein extracts were prepared at 0, 2, 6, and 16 hours after glutamine withdrawal, and subjected to western blotting for phosphorylated p70S6K, S6, 4EBP1, and AKT or LC3.

(C) SF188 cells were transfected with siRNA for CS and control for 48 hours. Fresh DMEM with glutamine (6 mM) was replaced for another 16 hours. Intracellular acetyl-CoA levels were measured by LC-MS and normalized to cellular protein content.

(D) SF188 cells were transfected with siRNA for CS and control in the presence of 6 mM $^{13}$C5-glutamine for 48 hours. Cellular aspartate was quantified by GC-MS. The result was shown as the ratio of the signal between each species of aspartate and the spiked-in standard of D5-2-hydroxyglutarate (D5-2HG). “M+” denotes molecular weight shift due to the $^{13}$C labeling that was derived from glutamine.

The data in Figure S3 (A, C and D) are shown as mean +/- S.D., n=3, and the p-value was determined by using Student’s 2-tailed t-test.

**Supplemental Figure 4, related to Figure 5. Asparagine specifically rescues glutamine depletion-induced cell death.**

(A) SF188 cells with (green line) or without (blue line) constitutively overexpressed BCL-$X_L$ were cultured in DMEM without glutamine (-Q) for 48 hours. In addition, SF188 cells were also cultured in DMEM without glutamine but with asparagine (-Q+N, red line) for 48 hours. Cellular ATP levels were measured at 0, 24 and 48 hours and normalized to the total number of viable cells.

(B) SF188 cells were cultured in DMEM without glutamine (-Gln), leucine (-Leu), threonine (-Thr) or phenylalanine (-Phe) respectively. 4 mM asparagine (Asn) was added or not added to each amino acid-deficient medium for 48 hours. Apoptosis was measured by Annexin V staining. Glutamine was also added to the (-Gln) condition to serve as a control.

(C) SF188 cells were cultured in DMEM without glucose (-Glc) or complete DMEM with 10 μM camptothecin (+CPT) for 48 hours with or without asparagine (+Asn) at 4 mM. Cell death was measured by Annexin V staining. Cells grown in complete DMEM (+Glc) served as a control.

(D) Kelly, NBLS and SV40 transformed MEFs were plated in complete medium for 2 days, and then switched to glutamine-deficient medium with or without asparagine (4 mM) for 4 days. Cellular viability was measured by Annexin V staining. The p-values were determined by using Student's 2-tailed t-test.

(E) Comparison of nonessential amino acid levels in -Gln and -Gln+Asn (4 mM) conditions. The values are identical to those presented in Figure 5E and are all normalized to -Gln condition.

The data in Figure S4 are shown as mean +/- S.D., n=3.

**Supplemental Figure 5, related to Figure 6. Expression of asparagine synthetase (ASNS) correlates with poor prognosis in neuroblastoma patients.**

(A) Immunohistochemical staining for ASNS in primary tumors of neuroblastoma patients. Normal adrenal cortex was used as a negative control of the staining. One

case of favourable neuroblastoma and 2 cases of unfavourable neuroblastoma were shown in representative images.

(B) Comparison of ASNS staining in tumor tissues from favourable (n=77) and unfavourable (n=52) neuroblastoma patients.

(C) Comparison of ASNS staining in tumor tissues from, differentiated (n=29), poorly differentiated (n=68) and undifferentiated (n=8) neuroblastoma patients.

(D) Comparison of ASNS staining in tumor tissues from non MYC-amplified (n=119) and MYC-amplified (n=19) neuroblastoma patients. Data in (B, C and D) were shown as mean + S.E.M., p-values were determined by Student’s 2-tailed t-test.

| Pathway | -log(pValue) | |
|---|---|---|
| | Screen 1 | Screen2 |
| Translation _Regulation of EIF4F activity | 1.98E-04 | 2.74E-04 |
| Development_Role of Activin A in cell differentiation and proliferation | 1.36E-03 | 1.73E-03 |
| Translation_Insulin regulation of translation | 1.57E-03 | 2.00E-03 |
| Signal transduction_AKT signaling | 1.68E-03 | 2.14E-03 |
| Development_TGF-beta-dependent induction of EMT via RhoA, PI3K and ILK. | 2.04E-03 | 2.60E-03 |
| Development_PIP3 signaling in cardiac myocytes | 2.17E-03 | 2.76E-03 |
| Regulation of lipid metabolism_Insulin signaling:generic cascades | 2.17E-03 | 2.76E-03 |
| Development_Beta-adrenergic receptors regulation of ERK | 2.17E-03 | 2.76E-03 |
| Cytoskeleton remodeling_Cytoskeleton remodeling | 2.35E-03 | 3.20E-03 |
| Some pathways of EMT in cancer cells | 2.75E-03 | 3.49E-03 |
| Regulation of lipid metabolism_Insulin regulation of fatty acid metabolism | 1.29E-02 | 1.62E-02 |

**Supplemental Table 1, related to Figure 7. Pathway analysis of the hits defined by low threshold of the secondary screen.**

The table shows a list of 11 biological pathways significantly associated with 111 nominated genes that were defined based on the summary of method 1 and 2 as described in the extended experimental procedures. Canonical pathway analysis was done using Gene Go's Metacore software (www.genego.com/metacore.php). Threshold for statistical level of significance was determined at a p-value of < 0.05 with a false discovery (FDR) < 0.01.

| Amino Acids | Estimated Intracellular concentration in SF188 cells (mM) | Reported concentration in human plasma (mM) |
|---|---|---|
| **Asparagine** | 0.08 ± 0.04 | 0.079 ± 0.024 |
| **Glutamate** | 17.4 ± 2.7 | 0.087 ± 0.037 |
| **Aspartate** | 3.2 ± 1.0 | 0.019 ± 0.005 |
| **Proline** | 23.0 ± 1.3 | 0.293 ± 0.057 |
| **Alanine** | 0.89 ± 0.45 | 0.489 ± 0.126 |

**Supplemental Table 2. Comparison between the concentration of non-essential amino acids in SF188 cells and that in human plasma.** SF188 cells were grown in complete DMEM for 48 hours and briefly rinsed with PBS prior to the extraction by 80% methanol. To measure intracellular asparagine, we used LC-MS with a CBZ derivatization protocol. The total amount of intracellular asparagine was normalized to a spiked-in internal standard of isotope labeled asparagine ($^{13}C_{4,}{}^{15}N_{2}$-L-asparagine) of known quantity. To estimate the intracellular concentration, the total amount of asparagine was divided by packed cell volume that is calculated based on cell number and average cell size measured by Multisizer 4 particle analyzer (Beckman Coulter). To measure the other 4 non-essential amino acids (NEAAs), we used GC-MS with a standard TMCS derivatization as described in the Experiment Procedures. We spiked in incremental amounts of non-isotope labeled NEAAs, and confirmed linear regression of the integrated value of individual NEAAs and the spiked-in quantities. Based on the slope of the linear regression equation, we calculated intracellular total amount of each NEAAs, which was further divided by the estimated packed cell volume to obtain the intracellular concentration. The details of the experiments were included in the

Extended Experiment Procedures. The NEAAs concentrations in human plasma after food ingestion was reported (Stegink et al., 1991).

## EXTENDED EXPERIMENTAL PROCEDURES

### Media Preparation

All cell lines in this study were cultured in Dulbecco's Modified Eagle Medium (DMEM, 11965, Life Technologies), supplemented with 10% FBS (100-106, GEMINI), 100 units/ml penicillin and 100 ug/ml streptomycin (400-109, GEMINI) and 2 mM additional L-glutamine. Glutamine deficient medium was prepared by using DMEM without glutamine (11960, Life Technologies), supplemented with 10% dialyzed FBS (100-108, GEMINI) and 100 units/ml penicillin and 100 ug/ml streptomycin (400-109, GEMINI). L-Asparagine (A0884, Sigma) was added at 4 mM or as mentioned in figure legends. Cell permeable $\alpha$-ketoglutarate (34963-1, Sigma) was added at 5 mM with 10 mM Hepes (15630, Life Technologies). DMEM without individual essential amino acids, including leucine, theronine or phenylalanine was prepared by the Media Preparation Core at MSKCC based on the standard formulations of DMEM (http://www.lifetechnologies.com/us/en/home/technical-resources/media-formulation.8.html).

### Information of siRNAs and shRNA

Individual siRNAs for human *CS* (s3582, s3584), *MYC* (s9131), *BAX* (s1889), *PDH$\alpha$* (s10243), and *CHOP* (s3995, s3997) are from Life Technologies and were used at 20 nM final concentration. siBAX9 (one of the positive control siRNAs for the screen) was obtained from Qiagen (S102661897) and used at 20 nM. siRNA for human *ASNS* (J-009377-06, J-009377-08) and mouse *Cs* (J-059581-11) are from Thermo Scientific and were used at 40 nM final concentration. shRNA for human CS (SKI-RSI-195551) was

obtained from the RNAi Core at MSKCC, which was originally supplied by Sigma-Aldrich RNAi Consortium (TRC) libraries.

**Antibodies and Western Blotting**

Protein extract was prepared in RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris pH8.0) supplemented with 1 mM DTT, protease inhibitor cocktail (sc-29131, Roche) and phosphatase inhibitor cocktails 2 and 3 (P5726 and P0044, Sigma). Equal amount of total protein was separated on NuPAGE Bis-Tris Gel (Life Technology), transferred to nitrocellulose membrane and blocked in 5% non-fat milk in TBS supplemented with 0.2% Tween (TBST). Membranes were incubated with primary antibodies in 3% BSA diluted in TBST overnight at 4°C. Primary antibodies used were: cytochrome c (556433, BD Biosciences), BCL-$X_L$ (610746, BD Biosciences), Caspase-3 (#9662, Cell Signaling), VDAC (#4661, Cell Signaling), CS (GTX110624, GeneTex), PDH$\alpha$ (18068-1-AP, ProteinTech), ASNS (14681-1-AP, ProteinTech), ATF4 (sc-200, Santa Cruz), CHOP (sc-575, Santa Cruz), BAX (06-499, Millipore), c-MYC (sc-764, Santa Cruz), p-p70S6K, p70S6K, p-S6, S6, p-4EBP1, 4EBP1, p-AKT, ATK (9205, 9202, 2211, 2317, 9451, 9452, 9271 and 9272, Cell Signaling) , $\alpha$-tubulin (T6199, Sigma), $\beta$-actin (A5441, Sigma). Rabbit anti-LC3 polyclonal antibody was generated by Quality Control Biochemicals. Membranes were washed with TBST, incubated with horseradish-peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (NA934V and NA931V, GE Healthcare) at room temperature for 1 hour, and developed with ECL Western Blotting Detection Reagent (RPN2106, GE Healthcare).

**siRNA Screen of Human Druggable Genomic Library**

The *Silencer* Human Extended Druggable Genome siRNA Library V3 was originally obtained from Ambion (Now Life Technologies). This library covers 9102 genes in human genome, with 4 siRNA duplexes of each gene with 2 siRNAs pooled per well that were pre-spotted onto fifty two 384-well plates with white bottom to give a final concentration of 20 nM in 40 µL media. Plate 23 and 50 were not included into the screen, because those 2 plates contain few siRNAs. On each plate, wells I~P×20~24 are empty and were used to spot our negative control (siCon), transfection control (siDeath), positive control (siMYC and siBAX) plus control wells that were not starved. siRNA were mixed with Lipofectamine RNAiMAX in DMEM media for 20 minutes, and 5000 cells were added to each well and centrifuged at 1200 rpm for 5 minutes. Two days post-transfection, medium was replaced with DMEM without glutamine for 4 days. Glutamine was then added back at a final concentration of 6 mM for another 2 days to allow the live cells to recover and grow. 25 µL of CellTiter-Glo Luminescent Reagent (G7572, Promega) was added to each well, incubated at room temperature for 10 minutes. Luminescence was read by an Analyst HT (Molecule Devices). The screen was performed in duplicate. For plates 26 to 54 of the duplicate screen, the luminescence was recorded by an Envision Multimode Plate Reader due to a technical issue with the Analyst HT. The data from plate 20 of the duplicate screen was also lost. The raw values were used to calculate the robust z-score of each plate based on the equation: z = (Value - Median) / [(Quantile 3 – Quantile 1)×0.74]. Therefore, each gene contains 4 z-scores, except the genes on plate 20.

**Candidate Hits Nomination**

To prioritize genes, we define hits as genes that must have three z-scores $\geq 2$, with at least one $\geq 3$ or two z-scores from the same siRNA pool $\geq 3.5$. Using this strategy, we identified 119 candidate genes for further validation. For the secondary validation screen, we added BBC3, MYC and BAX as three independent candidate genes to the list and used at least 3 siRNA (Silencer® Select, Life Technologies) against each candidate gene with the same positive and negative controls under the same condition as the primary screen, except that a brief PBS rinse was performed when we switched cells to glutamine deficient medium. Luminescence pixel density of CellTiter-Glo assay was obtained with the LEADseeker Multimodality Imaging System (GE Healthcare). The nucleotide sequences of Silencer® Select siRNA for each individual gene are completely different from the siRNA sequences in the primary screen. We nominated hits by 2 selection methods with low ($\geq \mu_{Neg}+2\sigma_{Neg}$, method 1) and high ($\geq \mu_{BAX}-2\sigma_{BAX}$, method 2) thresholds. For nomination of each gene, we followed the 3 hierarchical steps: 1). Selection of siRNA above the threshold as active siRNA; 2). Selection of all siRNA corresponding to the gene targeted by the active siRNA; 3). If no significant difference between the score of active and inactive siRNA (<15% below the threshold, also known as breakpoint cutoff), we scored them all as positive hits. Citrate synthase was selected as the best nomination by method 2. For the pathway analysis shown in the supplemental table 1, we applied 111 genes based on the summary of nomination of both method 1 and 2.

## Molecular Cloning and Lentivirus-mediated Infection

Mouse citrate synthase (mCS) cDNA was obtained from OriGene, and sequenced to confirm the identity. The mCS cDNA was cloned into the EcoR and NotI sites of pCDH-CMV-MCS-EF1-Puro (CD510B-1, SBI System Biosciences) lentiviral vector. The mCS vector and packaging plasmids (pCMV-V-SVG, pRSV-Rev, and pMDLg/pRRE) were transfected into 293T cells by Fugene 6 (E2691, Promega) according to standard protocol. Lentiviral particles were harvested at 48 hours and applied to the target cells in the presence of 6 μg/mL polybrene for 24 hours. The infected cells were cultured in fresh medium for another 24 hours, and then selected in the presence of 2 μg/mL puromycin for 24 hours.

## RNA Extraction and Quantitative PCR Analysis

RNA was extracted by Trizol (15596-018, Invitrogen) according to standard protocol and dissolved in DEPC-treated water (AM9915G, Ambion). 2 μg RNA was used for reverse transcription with random hexamers (S03496, Applied Biosystems) following the standard protocol of SuperScript® III Reverse Transcriptase (18080-093, Invitrogen). The RT-PCR product was treated with RNase H for 20 minutes at 37°C to remove the RNA complementary to the cDNA, and the cDNA product was diluted 5 times in nuclease free water. 2 μl diluted cDNA was used for quantitative PCR assay with Taqman® Universal Master Mix II, With UNG (4426710, Applied Biosystems) and Taqman® Gene Expression Assay Probes (Life Technologies). The catalog numbers of each probe are listed below: CHOP Hs00358796_g1; TXNIP Hs01006900_g1; XBP1s

Hs03929085_g1; ASNS Hs04186194_m1; BiP Hs00607129_gH; HERPUD1 Hs01124269_m1; 18S RNA Hs99999901_s1 was used as internal control.

**Immunofluorescence Staining and Confocal Imaging**

SF188 cells were grown on Lab-Tek II chamber slide (154526, Thermo Fisher Scientific) to reach 70% confluence. For staining of citrate synthase, cells were incubated with MitoTracker Red (M7512, Invitrogen) at 100 nM for 30 minutes at 37°C. Then cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.25% Triton with 3% BSA in PBS and then stained with rabbit polyclonal CS antibody (GTX110624, GeneTex) at 4°C overnight. Cells were washed and incubated with Anti-rabbit Alexa Fluor 488 (A21206, Invitrogen) for 1 hour at room temperature. After final wash, cells were briefly air dried and mounted with Prolong® Gold antifade with DAPI (P36935, Life Technologies). To stain conformational active BAX, cells were grown in the medium 48 hours with 20 μM Q-VD (1170-3, Biovision) and then fixed with 4% paraformaldehyde, permeabilized with 0.1% saponin in 1% BSA. Cells were sequentially incubated with anti-BAX (6A7) (2281-MC-100, Trevigen), and then incubated with Alexa Flour 488-conjugated goat anti-mouse IgG secondary antibody (A11001, Invitrogen) and Hoechst 33258 (B2883, Sigma). All the images were obtained with a Leica TCS SP5-II confocal microscope.

**Mitochondria Fractionation**

To separate mitochondria from cytosol, $40\times10^6$ SF188 cells were harvested and resuspended in HIM buffer (200 mM Manitol, 68 mM Sucrose, 10 mM Hepes-KOH pH7.4 and 1 mM EGTA) with protease and phosphatase inhibitors. All the processes

were handled on ice or at 4°C. Cells were broken up with a glass douncer with at least 30 strokes. Intact cells and debris were removed by low speed centrifugation at 600 g for 10 minutes. The supernatant was collected and centrifuged at 7000 g for 10 minutes to obtain the pellet, which is the heavy membrane fraction enriched by mitochondria. The supernatant was centrifuged at 14000 rpm for 10 minutes to remove light membranes from the cytosol. For western blotting of cytochrome C, 5 µg mitochondrial protein and 30 µg cytosolic protein was loaded.

**Citrate Synthase Activity Assay**

SF188 cells were collected in lysis buffer (250 mM Surcose, 20 mM Tris-HCl pH7.2, 2 mM EDTA, 0.1% Triton) and homogenized by sonication with Bioruptor-300 (Diagenode) at 4°C with the maximal intensity for 30 seconds per cycle for 3 cycles. Soluble fraction was collected by centrifugation and equal volume was applied to the reaction in cuvette containing 0.15 mM 5,5′-Dithiobis(2-nitrobenzoic acid) (D8130, Sigma), 0.5 mM Oxaloacetic acid (O4126, Sigma), 0.3 mM Acetyl-CoA (A2181, Sigma) and 0.1% Triton. Absorbance at 412 nm was recorded for 5 minutes. Activity was calculated as $\Delta OD_{412nm}$ per minute, and then normalized to the collected cell numbers.

**NAD/NADH and ATP Levels Assay**

NAD/NADH levels were measured by EnzyChrom$^{TM}$ $NAD^+$/NADH assay kit (E2ND-100, BioAssay Systems). Briefly, $10^6$ cells were used according to manufacturer's standard protocol. 40 µL of sample extraction was mixed with 80 µL of reaction reagent, and incubated at room temperature for 15 minutes. Absorbance at 565 nm was recorded, NAD/NADH concentration was calculated based on a standard curve generated by NAD

standard provided in the kit. ATP levels were measured by CellTiter-Glo Luminescent Assay (G7570, Promega). Basically, the medium and the dead cells were removed by aspiration, and fresh medium was added to the same volume. Equal volume of reconstituted CellTiter-Glo substrate was mixed directly with the cells in the culture medium, and incubated at room temperature for 10 minutes. Luminescence was recorded, and ATP concentration was calculated based on an ATP standard curve generated by pure ATP and normalized to the number of viable cells.

**Tumor Samples**

All human tissue samples were obtained from the University of Pennsylvania following approval from the Institutional Review Board. Cases were de-identified and two independent pathologists reviewed all cases prior to analysis. Samples were contained in previously well-characterized tissue microarray sections (Venneti et al., 2013a; Winter et al., 2008). For the glioma studies, 55 samples were analyzed and consisted of 3 World Health Organization (WHO) grade I pilocytic astrocytomas, 5 WHO grade II diffuse astrocytomas, 10 WHO grade III anaplastic astrocytomas, 31 WHO grade IV glioblastomas and 3 normal adult brain cortical tissues (Venneti et al., 2013a). Neuroblastoma tissue micro arrays consisted of 123 neuroblastomas (34 differentiated, 89 poorly differentiated), 33 ganglioneuroblastomas (17 intermixed, 16 nodular) and 11 ganglioneuromas. All neuroblastoma samples were annotated with the age at diagnosis, disease stage, prognosis, degree of differentiation, International Neuroblastoma Staging System Risk Stratification (INSS risk), MYCN gene amplification status, mitosis-karyorrexhis index (MKI) and histopathologic classification according to the International Neuroblastoma Pathologic Classification (INPC) Children's Oncology Group (COG)

(Winter et al., 2008).

**Immunohistochemistry and Automated Scoring**

Immunohistochemical studies and automated blinded quantification were performed as previously described (Venneti et al., 2013b). In brief, immunostaining was performed using the Discovery XT processor (Ventana Medical Systems). Tissue sections were blocked for 30 minutes in 10% normal goat serum in 2% BSA in PBS. Sections were incubated for 5 hours with the rabbit polyclonal anti-ASNS (14681-1-AP, ProteinTech, concentration 0.5 µg/ml) antibody. Tissue sections were then incubated for 60 min with biotinylated goat anti-rabbit IgG (Vector labs, PK6101) at 1:200 dilution. Blocker D, Streptavidin- HRP and DAB detection kit (Ventana Medical Systems) were used according to the manufacturer instructions.

Automated scoring was performed by scanning each slide using a Pannoramic Flash 250 scanner (Perkin Elmer, Waltham MA) and viewed through the Pannormaic viewer software program (3D Histech, Waltham MA). JPEG images from each tumor core (circular area of 315 $cm^2$ corresponding to the entire core) were captured at 20X magnification by an individual blinded to the experimental design. JPEG images were then quantified using an automated analysis program with Matlab's image processing toolbox based on previously described methodology (Venneti et al., 2013b). The algorithm used color segmentation with RGB color differentiation, K-Means Clustering and background-foreground separation with Otsu's thresholding. To arrive at a score for any given sample, the number of extracted pixels were multiplied by their average intensity and represented as pixel units. The final score for a given case and marker

was calculated by averaging the score of two core tumor samples for each case.

**Estimation of Intracellular Concentration of Nonessential Amino Acids (NEAAs)**

To measure intracellular asparagine, we spiked in 0.525 nmole $^{13}C_{4,}{}^{15}N_{2}$-L-asparagine to a single collection of a sample equivalent to 2.28 µL packed cell volume. After nitrogen dry of the samples, CBZ derivatization was performed prior to LC-MS. Each sample was resuspended in 400 µL running solvent and then 40 µl of triethylamine was added and mixed by vortexing. For each sample, 8 µl of benzylchloroformate was added, and incubated at room temperature for 30 minutes.

To quantify intracellular amount of other NEAAs, we spiked in an incremental known quantity of individual non-isotope labeled NEAAs to each sample equivalent to 3.38 µL packed cell volume. Below is the list of each standard quantity in nanomoles, glutamate (0, 160, 320, 640), aspartate (0, 40, 80, 160), proline (0, 40, 80, 160) and alanine (0, 40, 80, 160).

**SUPPLEMENTAL REFERENCES**

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