**Cell Culture**

All cell lines were obtained from ATCC, except HEK293FT, which was obtained from Invitrogen. All cells were maintained at 37 °C with 5% CO2. Basal DMEM (Corning) supplemented with 10% fetal bovine serum (FBS, Omega Scientific), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), 1x non-essential amino acids (Corning, 25-025-Cl) and pyruvate (1 mM) was used for culture of HEK293FT, A549, H2009, MDAMB231, KP4, SW620, HCT116, HT29, VCAP, and 22RV1. Basal RPMI (Corning) supplemented with 10% fetal bovine serum (FBS, Omega Scientific), penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM), was used for culture of H2122, H460, A549, H1975, H358, THP1, DAUDI, H2172, K562, NK92, Ramos, Jurkat, OCIAML3 and H1792. For SILAC experiments, each cell line was passaged at least six times in SILAC RPMI (Thermo), which lacks L-lysine and L-arginine, and supplemented with 10% (v/v) dialyzed FBS (Gemini), penicillin, streptomycin, L-glutamine (as above), and either [13C6, 15N2]- L-lysine and [13C6, 15N4]-L-arginine (100 µg/mL each) or L-lysine and L-arginine (100 mg/mL each). Heavy and light cells were maintained in parallel and cell aliquots were frozen after six passages in SILAC media and stored in liquid nitrogen until needed. For proteomic, transcriptomic, and functional assays conducted across the cell line panel, cells were equilibrated with Basal DMEM (Corning) supplemented with 10% fetal bovine serum (FBS, Omega Scientific), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), 1x non-essential amino acids (Corning, 25-025-cl) and pyruvate (1 mM) for two passages prior to assay. For metabolomics experiments using labeled metabolites, basal DMEM (Corning) supplemented with dialyzed 10% fetal bovine serum (FBS, Omega Scientific), penicillin (100 U/ml), streptomycin (100 μg/ml), and either 2 mM L-Glutamine (15N- amide, Sigma, 98% isotopic purity), 2 mM L-Glutamine (13C5, Sigma 605166, 98% isotopic purity), 2 mM L-Glutamine (U-13C15N, Sigma 607983), 5 mM glucose (U-13C, Sigma 389374, 99% isotopic purity) or unlabeled controls at the same concentration. For experiments analyzing ammonia uptake, 0.75 mM 15NH4Cl (Sigma, 98% isotopic purity) was supplemented in the presence or absence of glutamine where indicated. For experiments analyzing pyrimidine salvage, 10 μg/mL 2-13C-1,3-15N2-Uridine (Toronto Research Chemicals, U829907, 98% isotopic purity) was supplemented in the presence of 2 mM glutamine. Where dialyzed FBS was used, 10 μg/mL Uridine was supplemented as indicated in the main text. All cell lines were routinely evaluated for mycoplasma contamination by PCR using abm kit (Cat. No. G238) according to manufacturer’s protocol and validated by whole genome sequencing in reference to variants reported in by.

**Western Blotting**

Samples separated by SDS-PAGE were transferred onto nitrocellulose membranes. Membranes were incubated in Intercept (PBS) Blocking Buffer (LICOR Bioscience, 927-70001) or BSA 5% in 0.1% PBS-Tween 0.1 for 1 h at room temperature. Membranes were incubated overnight at 4 °C with primary antibodies diluted in blocking buffer, washed three times for 15 min with PBS and incubated for 1 h at room temperature with relevant species of 680RD or 800CW IRDye conjugated secondary antibodies (LiCOR) diluted in blocking buffer (1:20,000 dilution). The antibodies and dilutions used in this work were: OXA1L (1:1,000, Invitrogen, PA5-92880 and PA5-42895), GDA (1:1,000, Invitrogen, PA5-53977), CDA (1:1000, Abcam, 560053), CPS1 (1:000, Invitrogen, PA5-76339), ASS1 (1:1,000, Invitrogen, PA5-86054), ARG1 (1:1000, Invitrogen, 711765), ZFHX3 (1:1000, Invitrogen, PA5-63709), UQCRC2 (1:1000, Novus, H00007385-A01), V5 (1:1000, Abnova, MAB9755), TP53 (1:1000, Invitrogen, MA5-012571), TP53(D-01) (1:1000, Invitrogen, MA5-012571), ATP1A1 (1:1000, Abcam, ab7671), DHODH (1:1000, Proteintech, 14877-1-AP), MT-CO1 (1:1000, Abcam, ab14705), MT-CO2 (1:1000, Invitrogen, A6404), MT-CO2 (1:1000, Abcam, ab1656) MT-ND1 (1:1000, Novus Biologicals, H00004535-A01), VDAC1 (1:1000, Abcam AB34726), GAPDH, TUBULIN, and HSP90 (1:5,000, GeneTex, GTX627408, GTX27291, and GTX101423).

**Generation of Stably Transduced Cell Lines**

Lentiviral vectors (~3 μg) were co-transfected with 2.25 μg psPAX2.0 plasmid (Addgene, catalog number: 12260) and 0.75 μg CMV VSV-G (Addgene, catalog number: 98286) packaging plasmid into 5 × 106 HEK293FT cells cultured on 10 cm plates in complete DMEM lacking antibiotic using the lipofectamine 2000 transfection reagent (Invitrogen). Plasmids were combined in 1 mL DMEM and mixed with 36 μL of lipofectamine reagent diluted in 1 mL DMEM and incubated for 30 minutes. DNA-Lipofectamine 2000 complexes were then added dropwise to 10 cm plates. ~16 hours post transfection, medium was exchanged with complete DMEM lacking antibiotics. Virus-containing supernatants were collected an additional 24 hours after medium exchange, filtered and aliquots were stored at -80 °C until use. The virus was used to infect target cells in 6-well plates in the presence of 6 μg/ml polybrene (Santa Cruz). 24 hours post-infection, fresh media was added to the target cells, which were allowed to recover for an additional 24 hours. Medium containing a predetermined concentration of the appropriate antibiotic for each cell line was then added, and transduced cells were selected for 3-7 days depending on the antibiotic used. Prior to viral transduction, virus was titered in each cell line to be transduced by crystal violet staining 5-7 days post antibiotic selection, using a predetermined concentration of the appropriate antibiotic puromycin, blasticidin, or geneticin. For generation of pLenti3.3TR expressing H460, H1792 and PC3 cells (referred to as H460‐3.3, H1792‐3.3, PC3‐3.3), virus was added at a MOI of 10. Clonal pLenti3.3TR expressing cells were selected by cloning cylinder and TetR expression was validated by western blotting with anti-Tetracyline Repressor antibody (MoBiTec, Göttingen, Germany, Cat. no. TET01). Tetracycline repressor expressing clones were then transduced with pLenti6.3 virus at an MOI of 1. Tetracycline inducible expression of genes of interest were assayed by western blotting after 24-48-hour exposure to 1 μg/mL tetracycline using anti-V5 antibody.

**Generation of Knockout Cell Lines and CRISPR Competition Assays**

HSV-control, PCNA, CPS1, and OXA1L gRNAs were cloned into U6-gRNA:ef1a-tGFP plasmid (Sigma-Aldrich, LV03) or U6-gRNA:ef1a-puro-2A-Cas9-2A-tGFP (Sigma-Aldrich, LV01) or purchased directly. For clonal knockout cell lines, virus produced from LV01 containing HSControl or target gRNA was added to H460 cells at an MOI of five and transduced cells were selected using puromycin containing medium. Individual clones were selected by cloning cylinder and knockout was validated by antibody and sanger sequencing using TIDE analysis1. The targeted genetic modifications in cell lines used in this study are summarized in Supplementary Table 7. For functional assays, at least three individual clones were evaluated from each gRNA. For competition assays, CAS9 expressing parental cell lines were generated for each cell line in the 10-cell line panel as described above using the CAS9BST plasmid (Sigma-Aldrich, CAS9BST) and blasticidin selection with a cell-line dependent concentration of blasticidin. CAS9 expression was validated by western blotting using anti-V5 tag antibody and CAS9 expressing cells were maintained in blasticidin supplemented medium. CAS9 expressing cells were plated on clear bottomed CellCarrier 96-well plates at 5000 cells per well in complete medium lacking phenol red/antibiotic and allowed to adhere overnight. The following day, 100 μL of titered gRNA virus was added to each well in the presence of 6 μg/ml polybrene to achieve ~50% transduction rate, which was validated by relative count of GFP expressing cells. 24 hours post transduction, plates were imaged on a CX5 high content imager using Thermo Scientific HCS Studio 4.0 Cell Analysis Software at 2x magnification using the 485/521 LED and brightfield light source.  GFP signal was used for autofocus and images were collected daily for five days post-transfection. Total GFP cell count was followed over time for each gRNA in triplicate and used to get a relative growth rate after normalization to HS-Control gRNAs. The essential gene PCNA was used as a positive control, which inhibited the growth of all cell lines tested.

HS-Control gRNA1: ACTGTTGACGGCGGCGATGT

HS-Control gRNA2: GCTGATACCGTCGGCGTTGG

PCNA gRNA1: CTACCGCTGCGACCGCAACC

PCNA gRNA2: GAGTATAAAATTGCGGATAT

PCNA gRNA3: AGCTGCACCAAAGAGACGT

PCNA gRNA4: CTCTGCAGGTTTACACCGC

CPS1 gRNA1: TTTTCATAGAGCTACCAATT

CPS1 gRNA2: CGTCTCAACATCTGAAACTC

CPS1 gRNA3: CCCTACCGTCTCCAGATCGA

OXA1L gRNA1: AATTGTAGCAGTGACTGCCAG

OXA1L gRNA2: ATGGCGATGGGACTAATGTG

OXA1L gRNA3: GTTTTCCAGTCGAATCAGAG

**Generation of Barcode Libraries and Barcoded Cell Lines**

Double stranded DNAs harboring random 20-mers were generated by hybridization and primer extension reaction on ice by mixing 6 μL of 10 μM oligo1, 6 μL of 10 μM oligo2, 10 μL of 10× PCR buffer without Mg2+, 3 μL of 50 mM MgCl2, 2 μL of 10 mM dNTP mix, 0.5 μL of Taq DNA polymerase, and 72.5 μL of H2O. Reactions were incubated at 94 °C for 5 min, cooled to 55 °C over the course of 10 minutes, and then heated at 72 °C for 30 minutes. Duplexes were precipitated by addition of 12.5 μL of 3 M sodium acetate and 250 μL of 100% ethanol followed by incubation overnight at -80 °C. Precipitated DNA was collected by centrifugation for 25 min at 20,000 × g, washed twice with cold 75% ethanol, and resuspended in 40 μL of H2O.  PLKO.1 vector (2 μg) was digested in 10 μL of CutSmart buffer, with 3 μL of Age-HF and 3 μL of EcoRI-HF in a 100 μL total reaction. Inserts were digested by mixing 40 μL of DNA duplexes, 20 μL of CutSmart buffer, 3 μL of Age-HF, 3 μL of EcoRI-HF, and 134 μL of H2O. Both reactions were incubated at 37 °C for 4 h. PLK0.1 digests were than purified by gel and DNA duplexes were precipitated with 200 μL of H2O and 400 μL of fresh Tris buffered phenol/chloroform/isoamyl alcohol (pH 8.0) for 20 minutes, and spun for 20 min at 20,000 × g. Liquid phase was transferred into a new tube, and 40 μL of 3 M sodium acetate and  1 mL of 100% ethanol was added and duplexes were precipitated overnight at -80 °C. DNA was collected by centrifugation for 25 min at 20,000 × g, washed twice with cold 75% ethanol, and resuspended in 10 μL of H2O. Ligation reactions were conducted by mixing 1 uL of precipitated duplex with 1 uL of a 25 ng/uL PLK0.1 digest, 1 μL of 10× T4 DNA ligase buffer, and 1 μL of T4 DNA ligase in a total volume of 10 μL and incubated for 4 hours at RT, followed by heat inactivation for 10 min at 65°C. Ligations were than transformed into STBL3, and plated onto ampicillin agar plates 10x10 cm and incubated overnight. Library preparation was validated by by PCR amplification and sanger sequencing of ten individual bacterial colonies by mixing 200 ng of DNA extracted from 5 mL bacterial culture with 2.5 μL of 10 μM oligo3, 2.5 μL of 10 μM oligo4, 10 μL of 5× Standard Phusion buffer, 1 μL of 10 mM dNTP mix, 0.5 uL Phusion polymerase and 31.5 μL of H2O and amplified using 35 cycles of the following sequence: 98°C 0:15, 98°C 0:10, 59°C 0:15, 72°C 0:30. PCR reactions were cleaned using a DNA clean and concentrator kit and submitted for sanger sequencing using oligo 5. After library validation, at least 106 colonies from specific duplex reactions were scraped into 1 mL LB and purified using a plasmid mini prep kit according to manufacturer’s instructions. Barcode libraries were packaged by transfecting low passage 293FT using PsPax2 and VSV-G plasmids using lipofectamine 2000. Virus was tittered by transducing H460 cells followed by selection with 2 μg/mL puromycin for two weeks and stained with crystal violet.  For final generation of barcoded H460 cells, 25% confluent H460 cells were transduced at an MOI of 0.05 in the presence of polybrene (6 μg/ml) in 6-well plates, followed by selection with 2 μg/mL puromycin for two weeks. Barcoded H460 cells were maintained in puromycin containing medium and passaged no more than ten times.

Oligonucleotide sequences

oligo1: 5′-ggaaaggacgaaacACCGGTT-3′

oliog2: 5′-cgagaattcNNNNNNNNNNNNNNNNNNNNAAAAAACCGGTgtttcgtcctttcc-3′

oligo3: 5′-ggaggcttggtaggtttaagaa-3′

oligo4: 5′-ggatctctgctgtccctgtaat-3′

oligo5: 5′-ggaggcttggtaggtttaagaa-3′.

pLKO.1 (Addgene 10878)

psPAX2 (Addgene 12260)

pMD2.G (Addgene 12259).

**Measurement of Growth Rate Inhibition Metrics**

Growth rate inhibition metrics were measured according to previously published procedures with minor differences16. Briefly, GFP or RFP labeled cells were plated on clear bottomed CellCarrier 96-well plates at 5000 cells per well in complete DMEM lacking phenol red and allowed to adhere overnight.  The following day cells were treated with compounds over a dose-response range and imaged every 24 hours on a CX5 high content imager using Thermo Scientific HCS Studio 4.0 Cell Analysis Software at 2x magnification using the 485/521 LED and brightfield light source.  GFP or RFP signal was used for autofocus. Both GFP/RFP and brightfield images were used to calculate cell counts using the cellprofiler script “Cell\_Count\_Time.cpproj”. Cell counts for each compound, concentration and relevant control treatment were measured in triplicate at four 24 hour intervals with each cell and control having ≥3 biological replicates. Normalization to untreated control, and calculation of growth rate inhibition metrics using sigmoidal curve fitting, and integration was calculated according to the following equations, and implemented with the python script “Growth\_Rate\_Inhibition\_Plot.py”.

In general, cell count values at each of the four time points were normalized to t0, and used to fit an exponential curve based on the equation 6, where x cell count at time t (c,t), a is the initial cell count at t0, GR is the exponential growth rate constant, and b is a constant used to shift the curve based on differences in initial cell count across experiments and biological replicates.

eq 6: [Equation]

Growth rate constants at each concentration k(c), were then normalized to the growth rate of untreated controls k(0) and used to calculate GR values via equation 8, and used to fit a sigmoidal curve to calculate the concentration of drug at which GR(c) = 0.5, GR50.

eq 6: [Equation]

To calculate GR values across a subset of timepoints, cell count values at two or more timepoints x(c,t+Δt) and x(c,t- Δt) were used to calculate GR values based on equation 7.

eq 6: [Equation]

In addition to using the time course cell count assay, endpoint measurement of ATP content using the celltiterglo assay was normalized to the doubling time of untreated control cells to calculate growth rate inhibition where indicated. The untreated doubling Td = ln(2)/k(0) was measured in independent experiments and GR values were calculated based on the following equation:

eq 7: [Equation]

The GR50 value is the concentration of drug at which GR(c) = 0.5. If the value for GR(c) does not reach 0.5, GR50 was set to the highest concentration of drug tested (Supplementary Table 1). The GRmax is the maximum effect of the drug at the highest tested concentration and lies between −1 and 1; a value of 0 corresponds to a fully cytostatic response, and a negative value corresponds to a cytotoxic response. For time course data, all metrics are evaluated at each time point individually. The area under the dose response curve (GRAUC), was calculated by integrating the dose–response curve over the range of tested concentrations using -1 (fully cytotoxic) as the bottom of the integral.

**Measurement of Proliferation Over Extended Time**

Adherent GFP labeled cell lines growing in log phase were trypsinized, counted, and plated onto 6 well dishes (Corning) in 2 mL complete DMEM and incubated overnight. Initial seeding density was chosen based on relative doubling time for each cell line, but in general was around 1000 cells/well. The following day, one plate of cells was counted to determine the initial cell number at the time of treatment. Cells were washed 2 times with 2 mL phosphate buffered saline (PBS) and 4 mL media premixed with the indicated compounds added. For comparison of proliferation in the presence of galactose versus glucose, DMEM lacking glucose was supplemented with 10 mM glucose or galactose, 10% dialyzed FBS, 1 mM sodium pyruvate, 50 μg/mL uridine, and 100 U/mL penicillin/streptomycin.  For all conditions, the seeding densities allowed exponential proliferation over the time course of the experiment. The plates were imaged every 24 hours on a CX5 high content imager using Thermo Scientific HCS Studio 4.0 Cell Analysis Software at 2x magnification using the 485/521 LED and brightfield light source. GFP signal was used for autofocus. Images were used to calculate cell count using the cellprofiler script “Cell\_Count\_Time.cpproj”. Cell counts for each compound, concentration and relevant control treatment were measured in triplicate at four 24 hour intervals and ≥3 biological replicates.  Cumulative doublings were calculated from cell count using the following formula:

eq. 8: Cumulative doublings = ∑ log2 (Ti cell count / Ti-1 cell count).

**Analysis of Viability (ATP) by CellTiter Glo**

Cells were plated on 96-well plates at concentrations optimized based on proliferation rate of each cell line in 200 μl of complete DMEM supplemented with 10% fetal bovine serum (FBS, Omega Scientific), L-glutamine (2 mM), 1x Non-essential amino acids and pyruvate (1 mM). 12 to 24 hours after plating, 1 μL of each compound dilution series were transferred to the plates using a multichannel pipette. This yielded final drug concentration range of 1 µM to 0.5 nM (10-point dose response assay) by 3-fold serial dilutions, and a final DMSO concentration of under 0.5%. The cell–compound mixtures were incubated for 72 hours. Following incubation, cell numbers were determined by measuring the amount of ATP per well using Cell Titer Glo (Promega). Luminescence/well was measured using using a Tecan, Infinite F500 plate reader following manufacturer’s instructions. For every cell line and plate, compounds were tested in triplicate and validated at multiple times across a three year timespan yielding additional replicate values. On all plates, wells containing vehicle only or the general cytotoxic antimitotic Paclitaxel (10 nM) were also included. Raw values were normalized to the mean of vehicle wells and where indicated, the mean of the Paclitaxel positive control. Dose-response curves were fit using a four-parameter sigmoidal variable slope model, using a lower bound of less than 50%. For cell lines where a decrease in 50% was not attained, but data were of high quality, we set the IC50 values to the highest concentration tested over the 10-point dose range, which was used to calculate correlation with proteomic, transcriptomic, or genomic datasets. Additionally, we calculated a parameter AUC representing the area under the relative viability curve, defined as the sum of measured responses (relative viability) at all tested concentrations of the drug. Hence, AUC = 9 corresponds to an inactive compound, whereas smaller AUC values correspond to higher drug activities in inhibiting cell proliferation and/or inducing cell death. When multiple replicates of data on a drug or cell line combination are available, we used medians of the dose-response parameters estimated across replicates for the statistical analysis.

**Statistical Analysis of Association of Dose-response Parameters and Quantitative Omics Datasets**

We assessed associations of each of the key dose-response parameters, log10 (EC50), log10 (IC50), Hill slope, Emax and AUC for the set of n = 4 drugs and m = 44 cell lines with copy number, transcript and proteomic data using a linear regression model weighted on concordance between the datasets. Only genes quantified across datasets were considered. We used Brown’s extension of the Fisher’s combined probability test17 to combine Benjamini and Hochberg corrected P-Values, which considers dependencies between datasets, and thus provides a conservative estimates of significance for genes that are supported by multiple similar omics datasets. The integrated input gene list was then ranked by decreasing significance and filtered using a lenient cut-off, designed to capture candidate genes with sub-significant signals while discarding the bulk of insignificant genes (unadjusted Brown P gene < 0.1). To calculate the weights for genes within this list, the mean and SD of the expression values across cell lines was compared using. This list is shown in Supplementary Table S2. To test whether the concordance between protein, transcript and copy number variation is related to a biological function, we used the RECON2 genome scale model, based on Flux balance analysis (FBA), to characterize genes significantly correlated with drug sensitivity using the COBRApy package (Supplementary Methods 7.5, Supplementary Table 5). We used Partial Least Squares regression to maximize the covariance between datasets and the PLS components were used to weight.