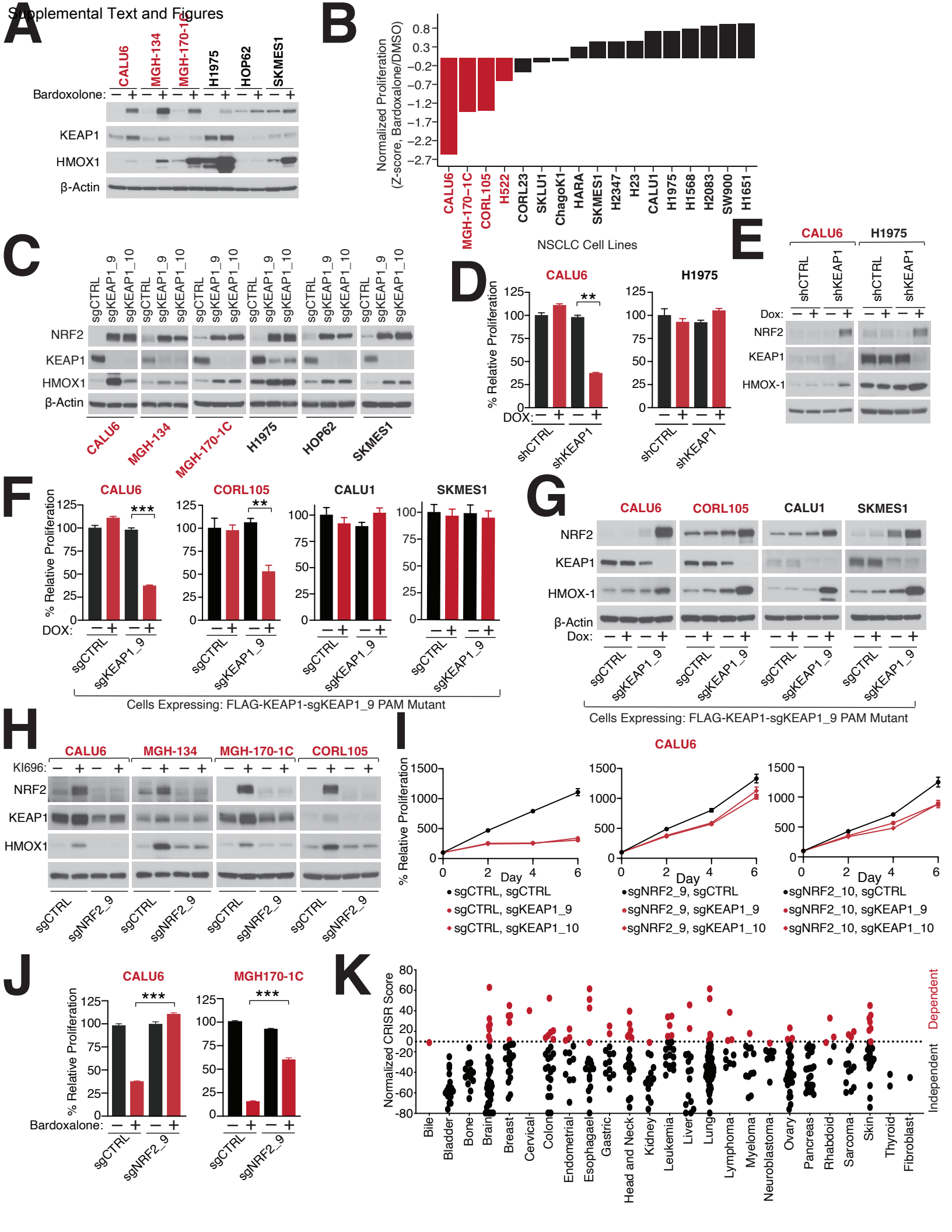
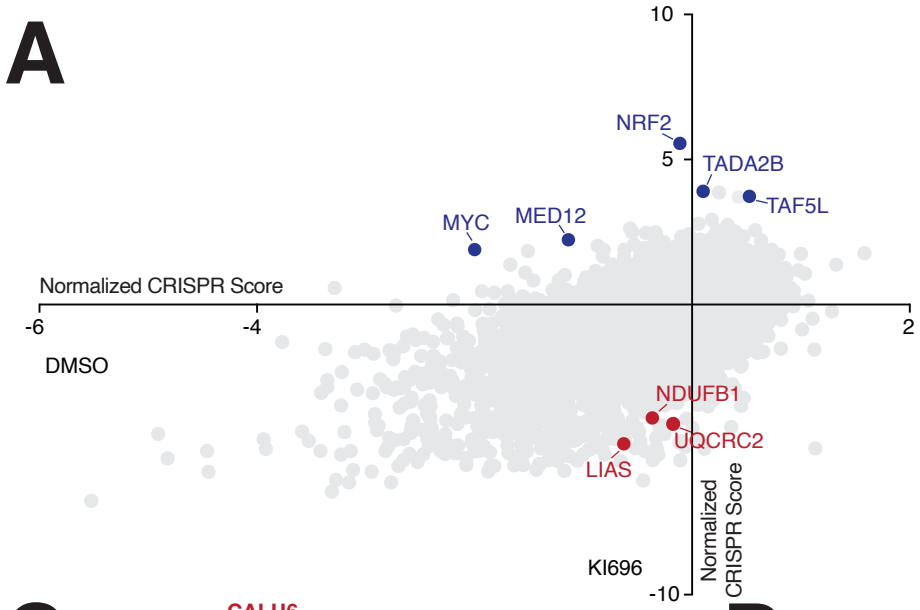
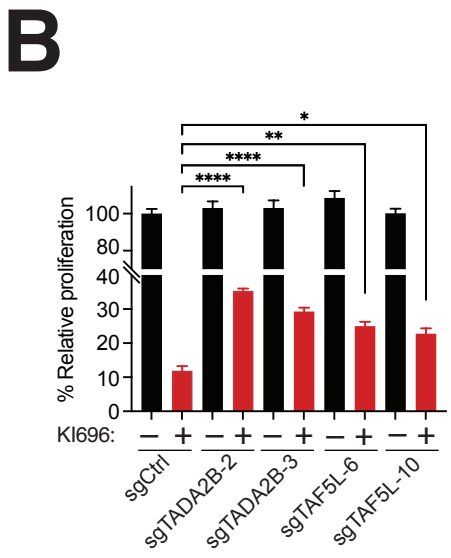
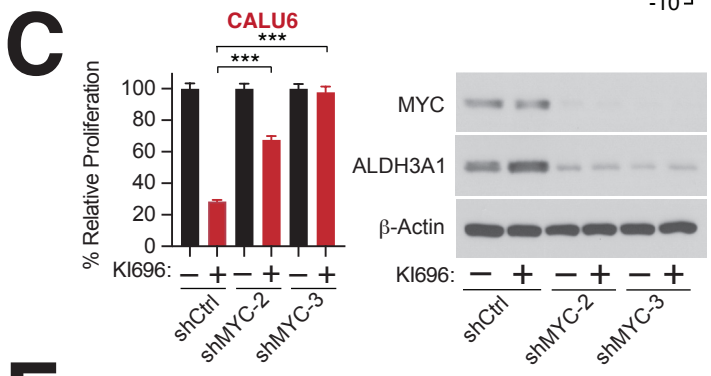

A
CALU6
MGH-134
MGH-170-1C
H1975
HOP62
SKMES1
Bardoxolone:
KEAP1
HMOX1
β-Actin
B
Normalized Proliferation (Z-score, Bardoxalone/DMSO)
CALU6
MGH-170-1C
CORL105
H522
CORL23
SKLU1
ChagoK1
HARA
SKMES1
H2347
H23
CALU1
H1975
H1568
H2083
SW900
H1651
NSCLC Cell Lines
C
sgCTRL
sgKEAP1_9
sgKEAP1_10
NRF2
KEAP1
HMOX1
β-Actin
D
% Relative Proliferation
DOX:
shCTRL
shKEAP1
E
Dox:
shKEAP1
HMOX-1
F
% Relative Proliferation
DOX:
sgCTRL
sgKEAP1_9
Cells Expressing: FLAG-KEAP1-sgKEAP1_9 PAM Mutant
G
Dox:
sgNRF2_9
sgNRF2_10
H
KI696:
sgNRF2_9
I
% Relative Proliferation
Day
sgCTRL, sgCTRL
sgCTRL, sgKEAP1_9
sgCTRL, sgKEAP1_10
sgNRF2_9, sgCTRL
sgNRF2_9, sgKEAP1_9
sgNRF2_9, sgKEAP1_10
sgNRF2_10, sgCTRL
sgNRF2_10, sgKEAP1_9
sgNRF2_10, sgKEAP1_10
J
% Relative Proliferation
Bardoxalone:
K
Normalized CRISR Score
Dependent
Independent
Bile
Bladder
Bone
Brain
Breast
Cervical
Colon
Endometrial
Esophageal
Gastric
Head and Neck
Kidney
Leukemia
Liver
Lung
Lymphoma
Myeloma
Neuroblastoma
Ovary
Pancreas
Rhabdoid
Sarcoma
Skin
Thyroid
Fibroblast

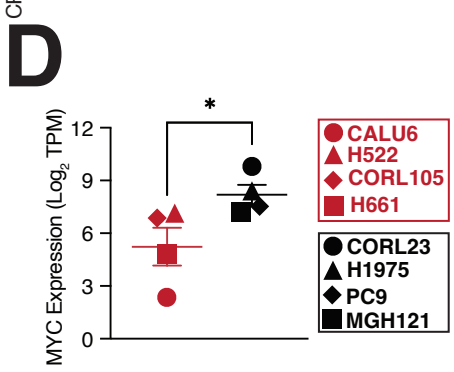
Figure S1: **Identification of KEAP1-dependent NSCLC cell lines, Related to Figure 1**. (A) Bardoxolone treatment activates NRF2. Representative immunoblot analysis of NSCLC cell lines treated with bardoxolone (100 nM) for 48 hrs. (B) Bardoxolone blocks the proliferation of KEAP1-dependent cells. NSCLC cells were treated with bardoxolone (100 nM) and proliferation was determined by crystal violet staining 6 days post-treatment. (C) Representative immunoblot analysis of NSCLC cell lines expressing the indicated sgRNAs targeting KEAP1. (D) shRNA-mediated depletion of KEAP1 blocks the proliferation of *KEAP1*-mutant cells. CALU6 and H1975 were infected with a doxycycline (DOX)-inducible shRNA targeting KEAP1 or a control. Cells were pre-treated with DOX (100 nM) for 72 hrs and cell proliferation was determined as described in (B) (Data are represented as a mean ± SEM, n= 3-5 biological replicates). (E) Representative immunoblot analysis of DOX-inducible depletion of KEAP1. (F-G) Addback of sgRNA resistant KEAP1 cDNA rescues KEAP1 depletion. NSCLC cell lines stably co-expressing a DOX-repressible FLAG-KEAP1-PAM mutant cDNA and sgRNAs targeting KEAP1 were treated with DOX (100 nM) for 72 hrs and cell proliferation (F) was determined as described in (B) and levels of the indicated proteins were determined by immunoblot (G) (Data for proliferation assay are represented as a mean ± SEM, n= 3-5 biological replicates). (H) Representative immunoblot analysis of NSCLC cell lines expressing the indicated sgRNAs targeting NRF2 following treatment with KI696 (1 μM). (I) Depletion of NRF2 rescues the proliferation of CALU6 cells following loss of KEAP1. The proliferation of CALU6 cells co-expressing sgRNAs targeting NRF2 or KEAP1 was assessed by measuring relative concentrations of ATP (Data are represented as a mean ± SEM, n=6 biological replicates). (J) NRF2-depletion rescues bardoxolone sensitivity in KEAP1-dependent cell lines. NSCLC cells expressing the indicated sgRNA targeting NRF2 or a control were treated with Bardoxolone and proliferation was determined as described in (B) (Data are represented as a mean ± SEM, n= 5 biological replicates). (K) KEAP1 is a dependency in multiple cancer subtypes as indicated in red. Cancer dependency (DEPMAP[1]) data was analyzed as described in [2]. * indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values <

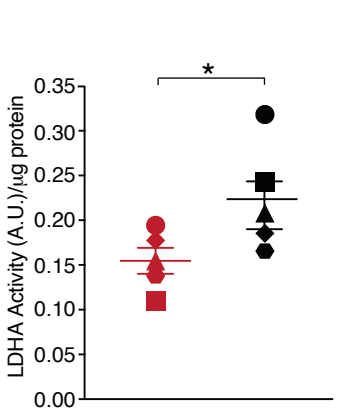
0.0001. One-way ANOVA with Sidak's post-hoc analyses used to determine statistical significance.







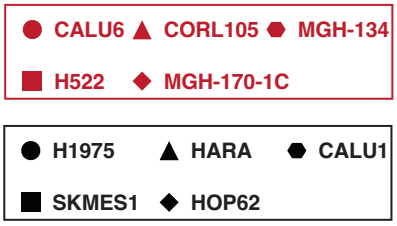


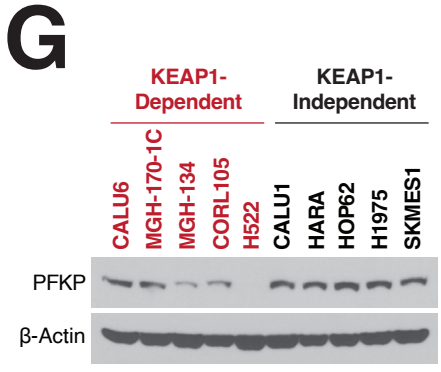


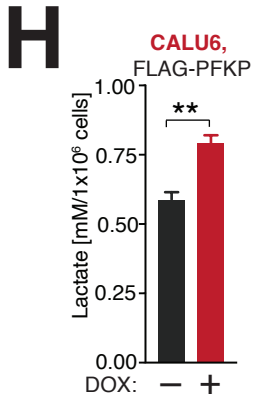
E

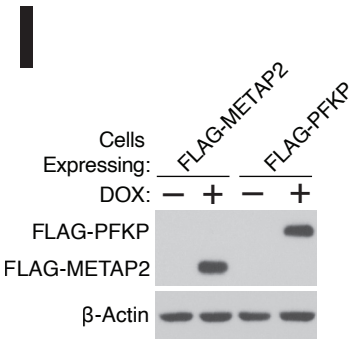
Metabolic CRISPR Screen

Pathway	CALU6 CRISPR Score (Log_2)	H1975 CRISPR Score (Log_2)	CALU6/H1975 Fold Change (Log_2)	p-value
OXPHOS	-0.6451	0.3275	**0.9726**	****
Sphinoglipid Metabolism	0.0660	0.0759	0.0099	NS
Phospholipid Metabolism	-0.7244	-0.5534	0.1710	NS
Amino acid Metabolism	-0.1115	-0.0369	0.0746	NS
Fatty acid Metabolism	0.0310	-0.0100	-0.0410	NS
Cholesterol Metabolism	-0.1108	-0.0207	0.1315	NS
Bile acid Metabolism	-0.0022	0.0531	0.0552	NS
Vitamin Metabolism	0.0059	0.0759	0.0700	NS
Glycolysis	0.5637	-0.305	**-0.8687**	****









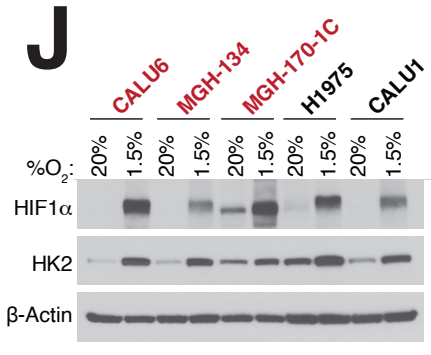


Figure S2: **KEAP1-independent cells are marked by high glycolytic rates, Related to Figure 1**. (A) Scatter plot of genome-wide CRISPR scores for individual genes in CALU6 cells treated with vehicle or KI696. Genes marked in red mediate sensitivity whereas genes marked in blue mediate resistance (see also **Figure 1E**). (B) Depletion of mediator components partially rescues proliferation following NRF2 activation. CALU6 cells expressing the indicated sgRNAs were treated with KI696 (1 μM) and proliferation was determined by crystal violet staining 6 days post treatment (Data are represented as a mean ± SEM, n= 30 biological replicates). (C) shRNA-mediated depletion of MYC rescues the proliferation of KEAP1-dependent cells following KI696 treatment. Right, CALU6 cells expressing the indicated shRNAs were treated with KI696 and proliferation was determined as described in (B). Left, immunoblot analysis of the indicated proteins in CALU6 cells expressing shRNAs targeting MYC and treated with KI696. (D) MYC has lower expression in KEAP1-dependent cells (data obtained from CBio Portal). (E) Table summarizing CRISPR scores for the indicated metabolic pathways in CALU6 and H1975 cells (see also **Figure 1H**). (F) KEAP1-dependent cells have lower lactate dehydrogenase (LDH) activity in comparison to KEAP1-independent cells. LDH activity was determined for each cell line as described in the methods (Data are represented as a mean ± SEM, n= 5 samples per group with 16 measurements per sample). (G) PFKP is differentially expressed in KEAP1-independent cell lines. Representative immunoblot analysis of the indicated proteins in KEAP1-dependent and KEAP1-independent NSCLC cell lines (see also **Figure 2C**). (H) Over-expression of PFKP increases lactate levels in CALU6 cells. CALU6 cells stably expressing DOX-inducible FLAG-PFKP were treated with DOX (100 nM) for 3 days and lactate levels were measured as described in the methods (Data are represented as a mean ± SEM, n= 3 biological replicates). (I) Immunoblot analysis of FLAG-PFKP or FLAG-METAP2 expression in CALU6 cells following DOX treatment (100 nM). (J) Induction of hypoxia in NSCLC cell lines. NSCLC cell lines were grown at the indicated oxygen concentrations for 3 days and the expression of the indicated proteins was

determined by immunoblot. * indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values < 0.0001. Statistical significance was determined by Student's t-test.

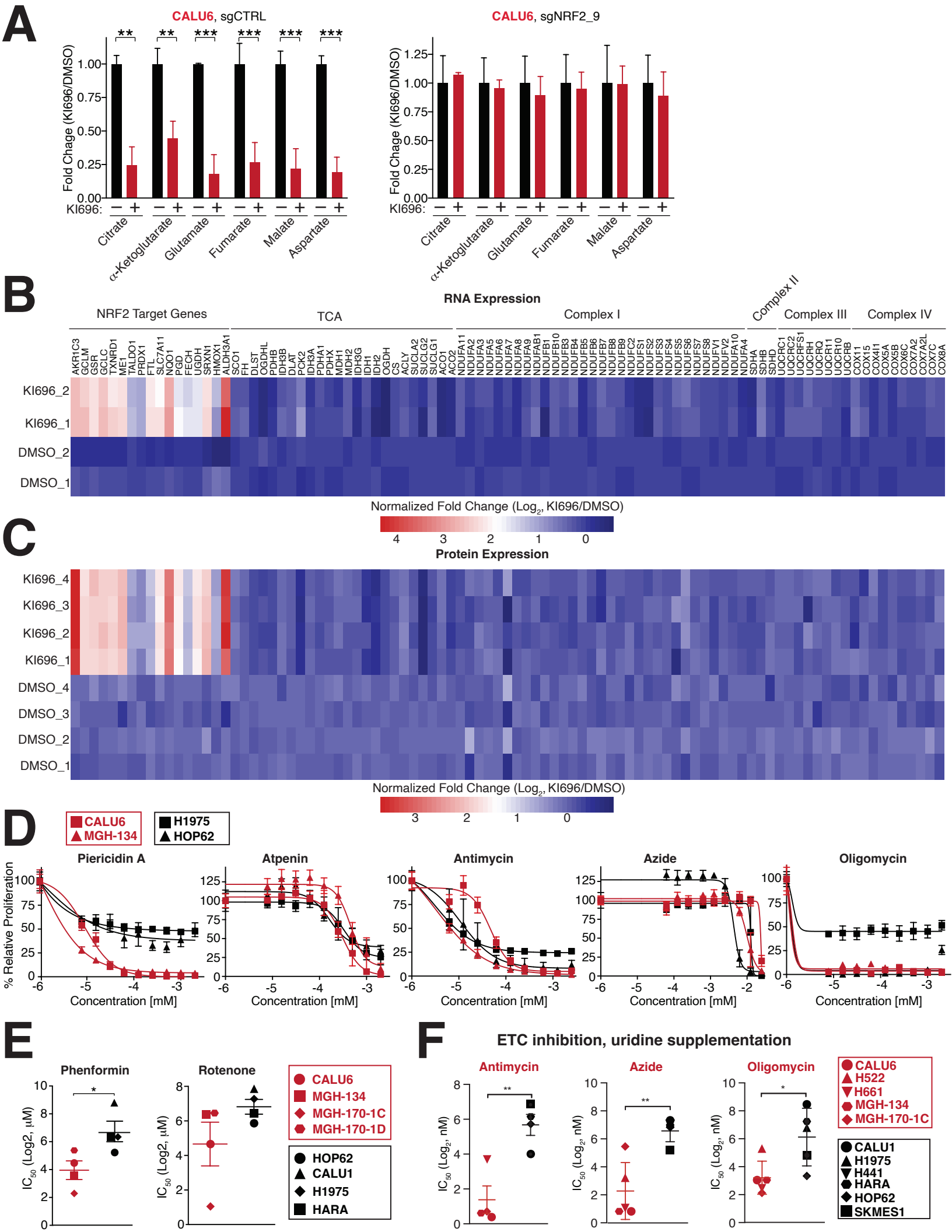

A
CALU6, sgCTRL
CALU6, sgNRF2_9
Fold Chage (KI696/DMSO)
KI696:
Citrate
α-Ketoglutarate
Glutamate
Fumarate
Malate
Aspartate
B
RNA Expression
NRF2 Target Genes
TCA
Complex I
Complex II
Complex III
Complex IV
KI696_2
KI696_1
DMSO_2
DMSO_1
Normalized Fold Change (Log2, KI696/DMSO)
C
Protein Expression
KI696_4
KI696_3
KI696_2
KI696_1
DMSO_4
DMSO_3
DMSO_2
DMSO_1
Normalized Fold Change (Log2, KI696/DMSO)
D
CALU6
MGH-134
H1975
HOP62
Piericidin A
Atpenin
Antimycin
Azide
Oligomycin
% Relative Proliferation
Concentration [mM]
E
Phenformin
Rotenone
IC50 (Log2, μM)
CALU6
MGH-134
MGH-170-1C
MGH-170-1D
HOP62
CALU1
H1975
HARA
F
ETC inhibition, uridine supplementation
Antimycin
Azide
Oligomycin
IC50 (Log2, nM)
CALU6
H522
H661
MGH-134
MGH-170-1C
CALU1
H1975
H441
HARA
HOP62
SKMES1

Figure S3: **KEAP1-dependent cells are sensitive to Complex I inhibition, Related to Figure 3**. (A) NRF2 regulates TCA metabolism in KEAP1-dependent cells. CALU6 cells expressing the indicated sgRNA targeting NRF2 or a non-targeting control, were treated with KI696 and the levels of m/z-values corresponding to the indicated metabolites were determined by LCMS (see methods). Fold change (KI696/DMSO) is depicted in the plots (Data are represented as a mean ± SEM, n=3 biological replicates). (B-C) NRF2 activation does not change the expression of mitochondrial genes involved in ETC or TCA cycle. CALU6 cells were treated with KI696 (1 μM) for 48 hrs and changes in gene expression (B) were determined by RNAseq or protein expression (C) were determined by proteomics and depicted in the corresponding heatmaps (see methods and **Data S1**). (D) Proliferation of NSCLC cell lines following treatment with the indicated ETC inhibitors following 6 days of treatment were determined by crystal violet staining (Data are represented as a mean ± SEM, n= 6 biological replicates). (E) Complex I inhibitors selectively block KEAP1-dependent cell lines. IC_{50}-values were determined for a panel of NSCLC cell lines (Data are represented as a mean ± SEM, n= 4 samples per group and 6 biological replicates). (F) Uridine supplementation selectively sensitizes KEAP1-dependent cells to Complex III-V inhibition. IC_{50}-values were determined for a panel of KEAP1-dependent and KEAP1-independent NSCLC cell lines grown in media containing uridine. Proliferation was determined as described in (D) (Data are represented as a mean ± SEM, n= 4 samples per group and 6 biological replicates* indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values < 0.0001. Statistical significance was determined by Student's t-test.

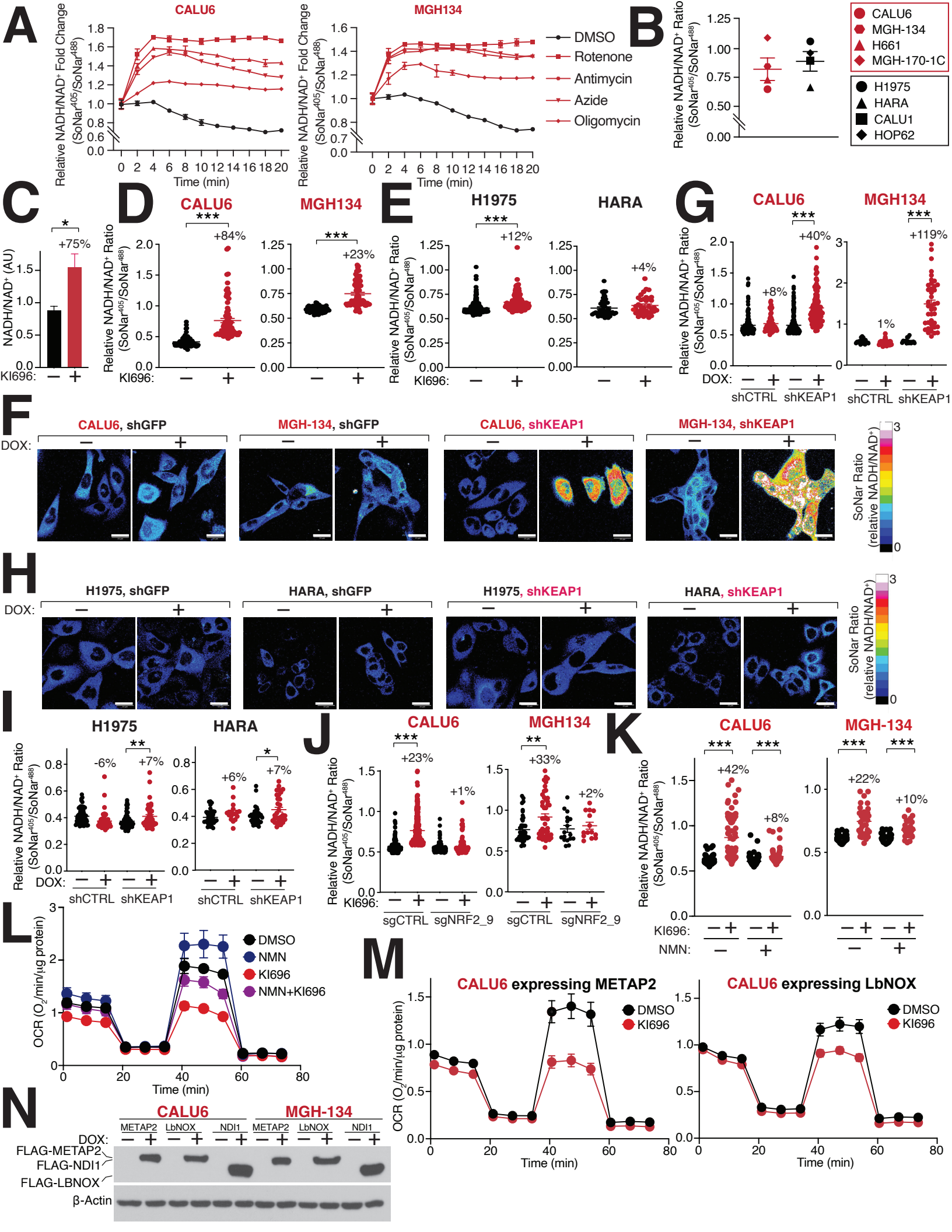

A
CALU6
MGH134
Relative NADH/NAD+ Fold Change (SoNar405/SoNar488)
Time (min)
DMSO
Rotenone
Antimycin
Azide
Oligomycin
B
Relative NADH/NAD+ Ratio (SoNar405/SoNar488)
CALU6
MGH-134
H661
MGH-170-1C
H1975
HARA
CALU1
HOP62
C
NADH/NAD+ (AU)
+75%
KI696:
D
CALU6
MGH134
+84%
+23%
E
H1975
HARA
+12%
+4%
G
CALU6
MGH134
+8%
+40%
1%
+119%
DOX:
shCTRL
shKEAP1
F
CALU6, shGFP
MGH-134, shGFP
CALU6, shKEAP1
MGH-134, shKEAP1
SoNar Ratio (relative NADH/NAD+)
H
H1975, shGFP
HARA, shGFP
H1975, shKEAP1
HARA, shKEAP1
I
H1975
HARA
-6%
+7%
+6%
+7%
J
CALU6
MGH134
+23%
+1%
+33%
+2%
sgCTRL
sgNRF2_9
K
CALU6
MGH-134
+42%
+8%
+22%
+10%
NMN:
L
OCR (O2/min/µg protein)
DMSO
NMN
KI696
NMN+KI696
Time (min)
M
CALU6 expressing METAP2
CALU6 expressing LbNOX
N
CALU6
MGH-134
METAP2
LbNOX
NDI1
FLAG-METAP2
FLAG-NDI1
FLAG-LBNOX
β-Actin

Figure S4: **NRF2 regulates the NADH/NAD$^+$ ratio in KEAP1-dependent cells, Related to Figure 4**. (A) ETC inhibition increases NADH/NAD$^+$ ratio. CALU6 and MGH134 cells expressing SoNar were treated with the indicated inhibitors and the NADH/NAD$^+$ ratio was determined by flow cytometry with the ratio of the emission intensity at λem 530nm after excitation λex at 405 nm (NADH binding) compared to excitation λex at 488 nm (NAD$^+$ binding). (B) KEAP1-dependent and KEAP1-independent cells have a similar NADH/NAD$^+$ ratio at basal states. The NADH/NAD$^+$ ratio in NSCLC cell lines stably expressing SoNar was determined as described in (A) (Data are represented as a mean ± SEM, n= 4 samples per group). (C) NRF2 activation increases the NADH/NAD$^+$ ratio in CALU6 cells. CALU6 was treated for 2 days with KI696 (1 μM) and the NADH/NAD$^+$ ratio was determined using a coupled enzymatic method as described in **Methods** (Data are represented as a mean ± SEM, n= 3 biological replicates). (D) Quantification of NADH/NAD$^+$ ratio following NRF2 activation in KEAP1-dependent cells. NSCLC cell lines expressing SONAR were treated for 2 days with KI696 and the NADH/NAD$^+$ ratio was determined by immunofluorescence analysis comparing the emission intensity after excitation at λex 405 nm to λex 488nm (Data are represented as a mean ± SEM, n=138 cells for MGH-134 and n=162 cells for CALU6 were analyzed from 2 biological replicates) (see also **Figure 4A**). (E) Quantification of NADH/NAD$^+$ ratio following NRF2 activation in KEAP1-independent cells. Cells stably expressing SONAR were treated and analyzed as described in (D) (Data are represented as a mean ± SEM, n=156 cells for H1975 and n=138 cells for HARA were analyzed from 2 biological replicates) (see also **Figure 4A**). (F-G) Depletion of KEAP1 in CALU6 or MGH-134 increases NADH/NAD$^+$ ratio. NSCLC cell lines stably expressing SoNar and a DOX-inducible shRNA targeting KEAP1 or a non-targeting control were treated with DOX (100 nM) for 3 days and the NADH/NAD$^+$ ratio was quantified (G) as in (D) (Data are represented as a mean ± SEM, n=232 cells for CALU6 and n=160 cells for MGH-134 from 2 biological replicates). (H-I) Depletion of KEAP1 in H1975 or HARA only moderately increases the NADH/NAD$^+$ ratio. NSCLC cells stably co-expressing SoNar and indicated DOX-inducible shRNAs (H) were treated and analyzed (G) for changes in

NADH/NAD$^+$ ratio as described in (F-G) (Data are represented as a mean ± SEM, n=204 cells for H1975 and n=156 cells for HARA were analyzed from 2 biological replicates). (J) NRF2 regulates the NADH/NAD$^+$ ratio in KEAP1-dependent cells. Quantification of NADH/NAD$^+$ in NSCLC cell lines expressing the indicated sgRNAs and treated with KI696 (1 μM) for 2 days (Data are represented as a mean ± SEM, n=284 cells for CALU6 and n=184 cells for MGH-134 were analyzed from 2-3 biological replicates) (see also **Figure 4B**). (K) NMN treatment decreases the NADH/NAD$^+$ ratio following NRF2 activation. NSCLC cell lines expressing SoNar were co-treated with NMN (1 mM) and KI696 (1 μM) for 2 days and the NADH/NAD$^+$ ratio was determined as described in (A) (Data are represented as a mean ± SEM, n=213 cells for CALU6 and n=198 cells for MGH-134 were analyzed from 2 biological replicates) (**see also Figure 4C**). (L) NMN treatment rescues NRF2-mediated blockage of respiration. CALU6 cells were co-treated with NMN (1 mM), KI696 (1 μM) for 2 days or vehicle controls and OCR was determined by Seahorse Bioflux Analyzer (Data are represented as a mean ± SEM, n=6-8 biological replicates). (M) Over-expression of LBNOX overcomes NRF2-mediated blockage in OCR. CALU6 cells stably expressing DOX-inducible LbNOX or METAP2 (control) were pre-treated with DOX (100 nM) for 2 days followed by KI696 (1 μM) for another 2 days and OCR was determined as described in (L) (Data are represented as a mean ± SEM, n=6-8 biological replicates). (N) Representative immunoblot analysis of CALU6 and MGH-134 cells stably expressing DOX-inducible LbNOX, NDI1 or METAP2 following DOX treatment for 2 days. * indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values < 0.0001. Statistical significance was determined by Student's t-test or one-way ANOVA with Sidak's correction for multiple hypotheses for (G-J). Scale bar: 25 μm.

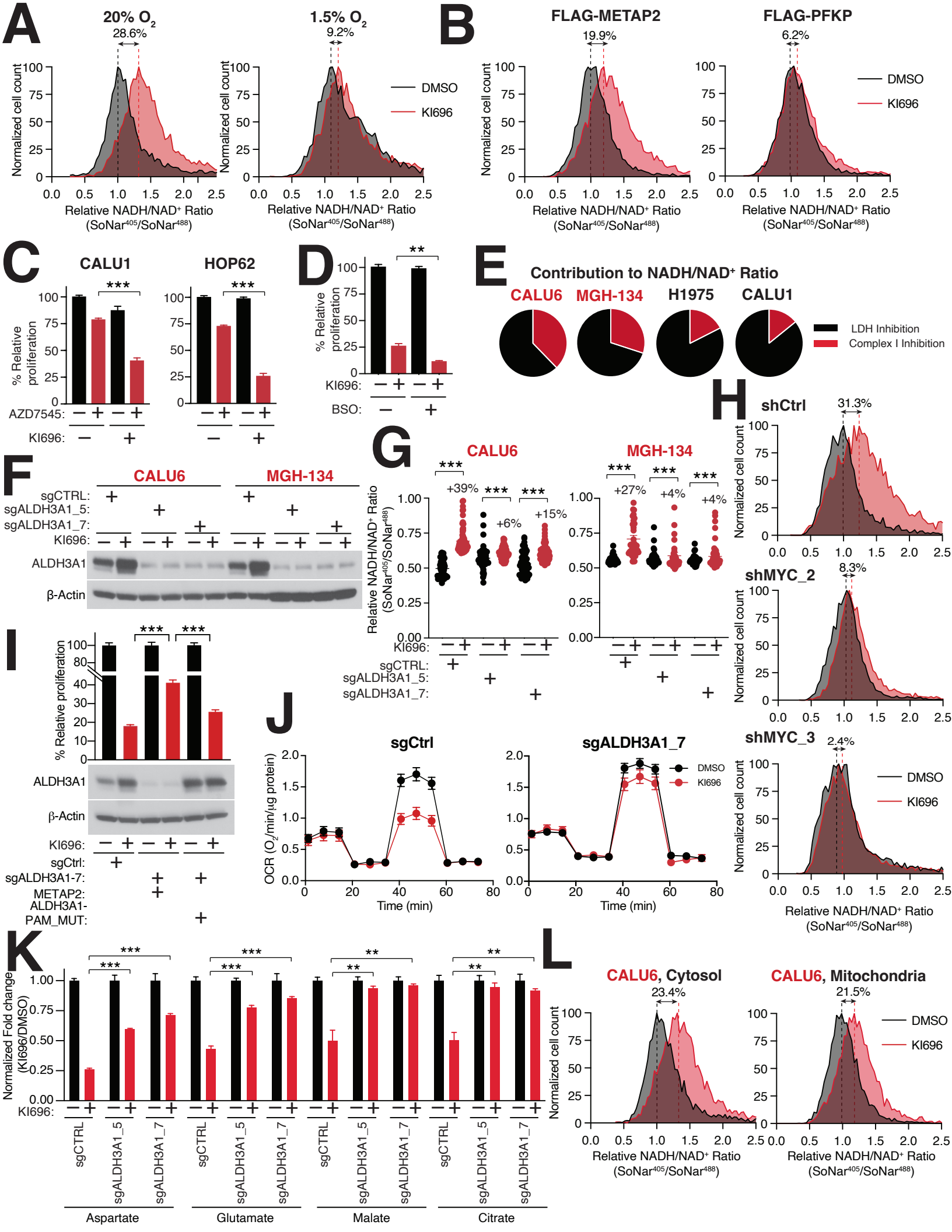

A
20% O2
28.6%
1.5% O2
9.2%
DMSO
KI696
Normalized cell count
Relative NADH/NAD+ Ratio
(SoNar405/SoNar488)
B
FLAG-METAP2
19.9%
FLAG-PFKP
6.2%
C
CALU1
HOP62
% Relative proliferation
AZD7545:
KI696:
D
BSO:
E
Contribution to NADH/NAD+ Ratio
CALU6
MGH-134
H1975
CALU1
LDH Inhibition
Complex I Inhibition
F
CALU6
MGH-134
sgCTRL:
sgALDH3A1_5:
sgALDH3A1_7:
ALDH3A1
β-Actin
G
Relative NADH/NAD+ Ratio (SoNar405/SoNar488)
+39%
+6%
+15%
+27%
+4%
+4%
H
shCtrl
31.3%
shMYC_2
8.3%
shMYC_3
2.4%
I
sgCtrl:
sgALDH3A1-7:
METAP2:
ALDH3A1-PAM_MUT:
J
sgCtrl
sgALDH3A1_7
OCR (O2/min/µg protein)
Time (min)
K
Normalized Fold change (KI696/DMSO)
Aspartate
Glutamate
Malate
Citrate
L
CALU6, Cytosol
23.4%
CALU6, Mitochondria
21.5%

Figure S5: **ALDH3A1 is a key regulator of the NADH/NAD$^+$ ratio following NRF2 activation, Related to Figure 5**. (A) Hypoxia decreases NADH/NAD magnitude change upon NRF2 activation. CALU6 cells expressing SoNar were grown under normoxic or hypoxic conditions for 3 days, prior to 48 hr treatment with KI696 (1 μM). NADH/NAD ratio was determined by flow cytometry analysis comparing the emission intensity after excitation at 405 nm and 488 nm. (B) Over-expression of PFKP stymies NRF2-mediated NADH increase. CALU6 cells stably expressing SoNar and DOX-inducible FLAG-PFKP or FLAG-METPA2 (control) were treated with DOX (100 μM) for 48 hrs and then KI696 (1 μM) for an additional 48 hrs. NADH/NAD ratio was determined by flow cytometry as described in (A). (C) Inhibition of pyruvate dehydrogenase kinase (PDK) sensitizes KEAP1-independent cells to NRF2 activation. NSCLC cell lines were co-treated with AZD7545 (PDK inhibitor, 100 μM) and KI696 (1 μM) for 6 days. Cell proliferation was determined by crystal violet staining (Data are represented as a mean ± SEM, n= 5 biological replicates). (D) Inhibition of glutathione synthesis does not rescue NRF2 activation in KEAP1-dependent cell lines. CALU6 cells were co-treated with BSO (50 μM) or KI696 (1 μM) and proliferation was determined as described in (C) (Data are represented as a mean ± SEM, n= 5 biological replicates). (E) KEAP1-dependent cells have a greater reliance on Complex I for NADH oxidation. NSCLC cell lines expressing SoNar were treated with rotenone (Complex I inhibitor, 0.5 μM) followed by treatment with oxamate (LDHA inhibitor, 5 mM) and the change in NADH/NAD$^+$ ratio was as described in (A). NADH/NAD$^+$ ratio was normalized to the max ratio observed following treatment with both inhibitors (Data are represented as a mean ± SEM, n= 2 samples per group representative of two biological replicates). (F) Representative immunoblot of ALDH3A1 in CALU6 and MGH-134 cells expressing sgRNAs targeting ALDH3A1. (G) Depletion of ALDH3A1 in KEAP1-dependent cells reverts NRF2 mediated increase in NADH/NAD$^+$ levels. NSCLC cell lines co-expressing SoNar and the indicated sgRNAs were treated with and KI696 for 2 days and the NADH/NAD$^+$ ratio was determined by immunofluorescence analysis comparing the emission intensity after excitation with 405 nm and 488 nm (Data are represented as a mean

± SEM, n=429 cells for CALU6 and n=286 cells for MGH-134 were analyzed from 4 biological replicates) (see also **Figure 5B**). (H) Depletion of MYC reverts NADH/NAD^+ following NRF2 activation. CALU6 cells co-expressing SoNar and the indicated shRNAs were treated with KI696 (1 μM) for 48 hrs and the NADH/NAD^+ ratio was determined by flow cytometry as described in (A). (I) Expression of a PAM-mutant ALDH3A1 restores KI696-sensitivy in KEAP1-dependent cells. CALU6 cells co-expressing ALDH3A1 PAM-mutant or control protein (METAP2) along with sgRNA targeting ALDH3A1 were treated with KI696 (1 μM) and proliferation was determined as in (C). (J) ALDH3A1 depletion partially rescues NRF2-mediated defects in respiration. CALU6 cells expressing the indicated sgRNAs were treated with KI696 (1 μM) for 48 hrs and respiration was determined by Seahorse Bioflux Analyzer (Data are represented as a mean ± SEM, n=8 biological replicates). (K) Reducing ALDH3A1 levels restores TCA metabolites after NRF2-activation. CALU6 cells expressing the indicated sgRNAs were treated with KI696 (1 μM) for 48 hrs and the levels of the indicated metabolites were determined by GCMS (see methods). Fold change (KI696/DMSO) is depicted in the plots (Data are represented as a mean, n= 3 biological replicates per sample). (L) Activating NRF2 increases NADH/NAD^+ ratio in the cytosol and mitochondrial matrix. CALU6 and MGH134 cells expressing cytosolic and mitochondrial matrix SoNar were treated for 48 hrs with KI696 (1 μM) and the change in NADH/NAD^+ ratio was determined as described in (A). * indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values < 0.0001. Statistical significance was determined by one-way ANOVA with Sidak's post-hoc correction.

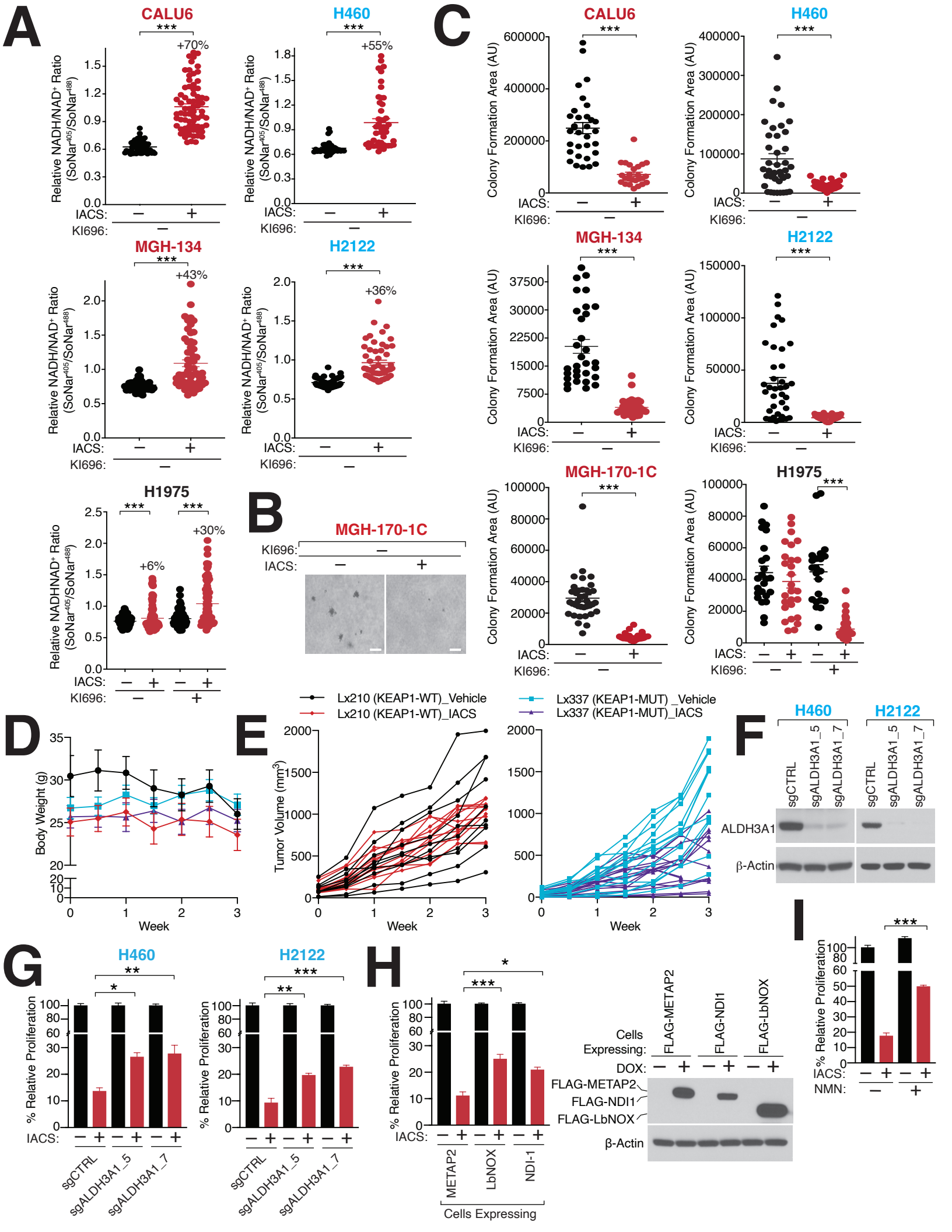

A
CALU6
H460
MGH-134
H2122
H1975
Relative NADH/NAD+ Ratio (SoNar405/SoNar488)
+70%
+55%
+43%
+36%
+6%
+30%
IACS:
KI696:
B
MGH-170-1C
C
Colony Formation Area (AU)
D
Body Weight (g)
Week
E
Lx210 (KEAP1-WT)_Vehicle
Lx210 (KEAP1-WT)_IACS
Lx337 (KEAP1-MUT) _Vehicle
Lx337 (KEAP1-MUT)_IACS
Tumor Volume (mm3)
F
sgCTRL
sgALDH3A1_5
sgALDH3A1_7
ALDH3A1
β-Actin
G
% Relative Proliferation
H
METAP2
LbNOX
NDI-1
Cells Expressing
FLAG-METAP2
FLAG-NDI1
FLAG-LbNOX
DOX:
I
NMN:

Figure S6: **Increasing the NADH/NAD$^+$ ratio selectively blocks the growth of NRF2-activated cells, Related to Figure 6**. (A) IACS-010759 (IACS) increases the NADH/NAD$^+$ ratio in KEAP1-dependent and *KEAP1*-mutant but not KEAP1-independent cells. NSCLC cell lines expressing SONAR were co-treated with IACS (100 nM) and KI696 (1μM) and the NADH/NAD$^+$ ratio was determined by immunofluorescence analysis comparing the emission intensity following excitation at 405 nm to 488nm (Data are represented as a mean ± SEM, n=116 cells for CALU6, MGH-134, H460, and H2122, n=332 cells for H1975) (see also **Figure 6A**). (B) Representative images of MGH-170-1C cells grown in soft agar following treatment with IACS-017509 (200 nM). (C) Quantification of IACS treatment on NSCLC anchorage-independent growth. NSCLCs were co-treated with IACS (200 nM) and KI696 (1 μM) and soft-agar proliferation was determined by assessing colony forming area in multiple frames using ImageJ (Data are represented as a mean ± SEM, n= 4-6 biological replicates) (see also **Figure 6C**). (D) Body weight plot of mice harboring KEAP1-MUT or KEAP1-WT PDX during treatment duration with IACS or vehicle (see also **Figure 6D**). (E) Individual tumor volumes for KEAP1-WT (left) or KEAP1-MUT (right) PDXs treated with vehicle or IACS (see also **Figure 6D**). (F-G) Decreasing ALDH3A1 partially rescues IACS-010759 treatment in *KEAP1*-mutant cells. Immunoblot analysis of ALDH3A1 in H460 and H2122 cells expressing sgRNAs targeting ALDH3A1 (F). H460 and H2122 sgRNAs targeting ALDH3A1 and respective controls were treated with IACS or vehicle for 6 days and proliferation was determined by crystal violet staining (G). (Data are represented as a mean ± SEM, n= 6 biological replicates). (H) Increasing NADH oxidation decreases IACS toxicity. H460 cells expressing DOX-inducible LbNOX, ND1I or METAP2 (control) were treated with DOX (100 nM) for 48 hrs and proliferation (left) was determined following 6 days of IACS treatment as described in (G). Levels of indicated proteins were determined by immunoblot (right) (Data are represented as a mean ± SEM, n= 6 biological replicates). (I) NMN supplementation decreases IACS toxicity. H460 cells were treated with 1mM NMN for 48 hrs followed by treatment with IACS (500 nM) and proliferation was determined as described in (G) (Data are represented as a mean ± SEM, n= 6 biological

replicates).* indicates *p*-values < 0.05, ** indicates *p*-values < 0.01, *** indicates *p*-values < 0.0001. One-way ANOVA and Student's t-test were used to determine statistical significance. Scale Bar: 50 μm.

References

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