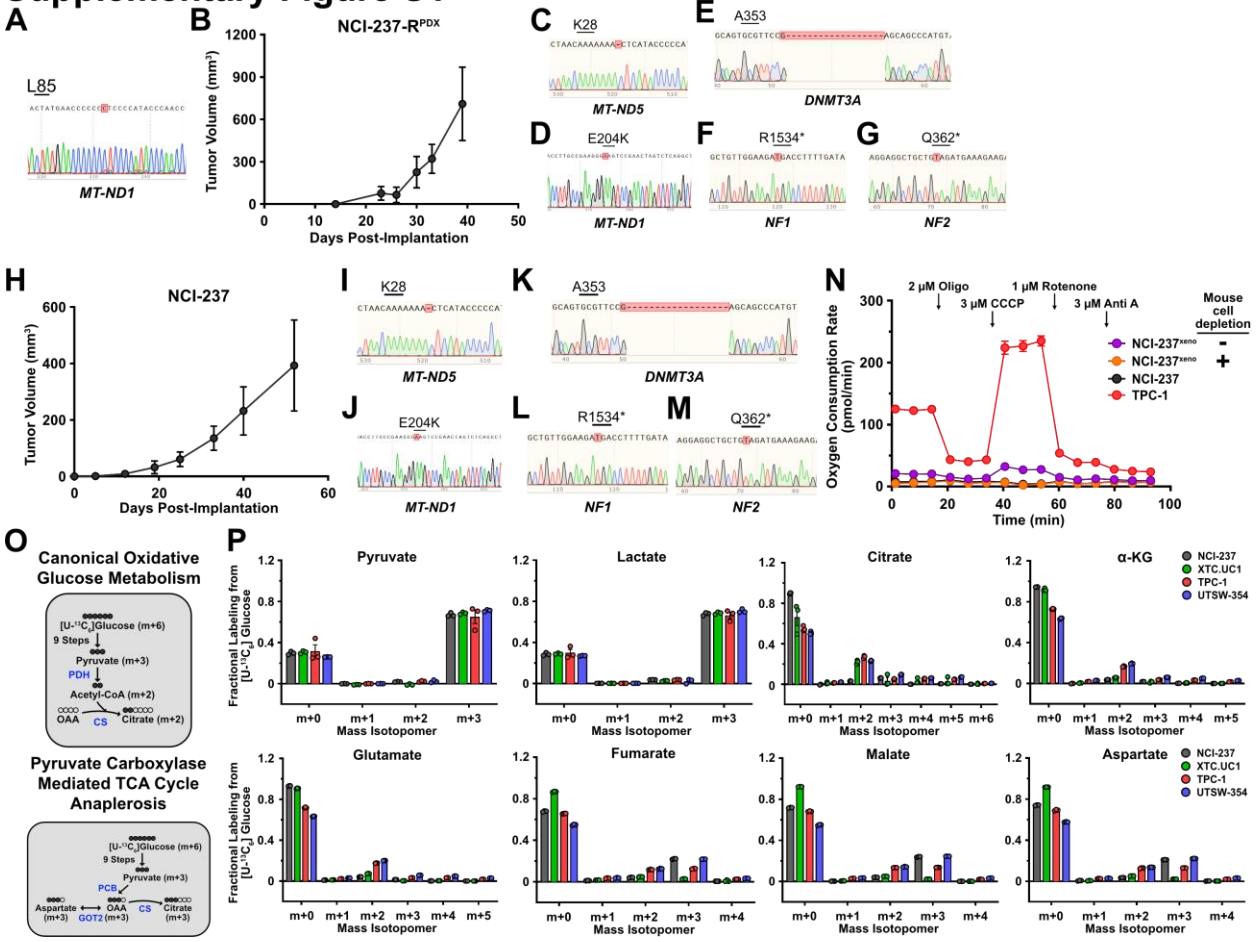
SUPPLEMENTARY FIGURE LEGENDS AND FIGURES

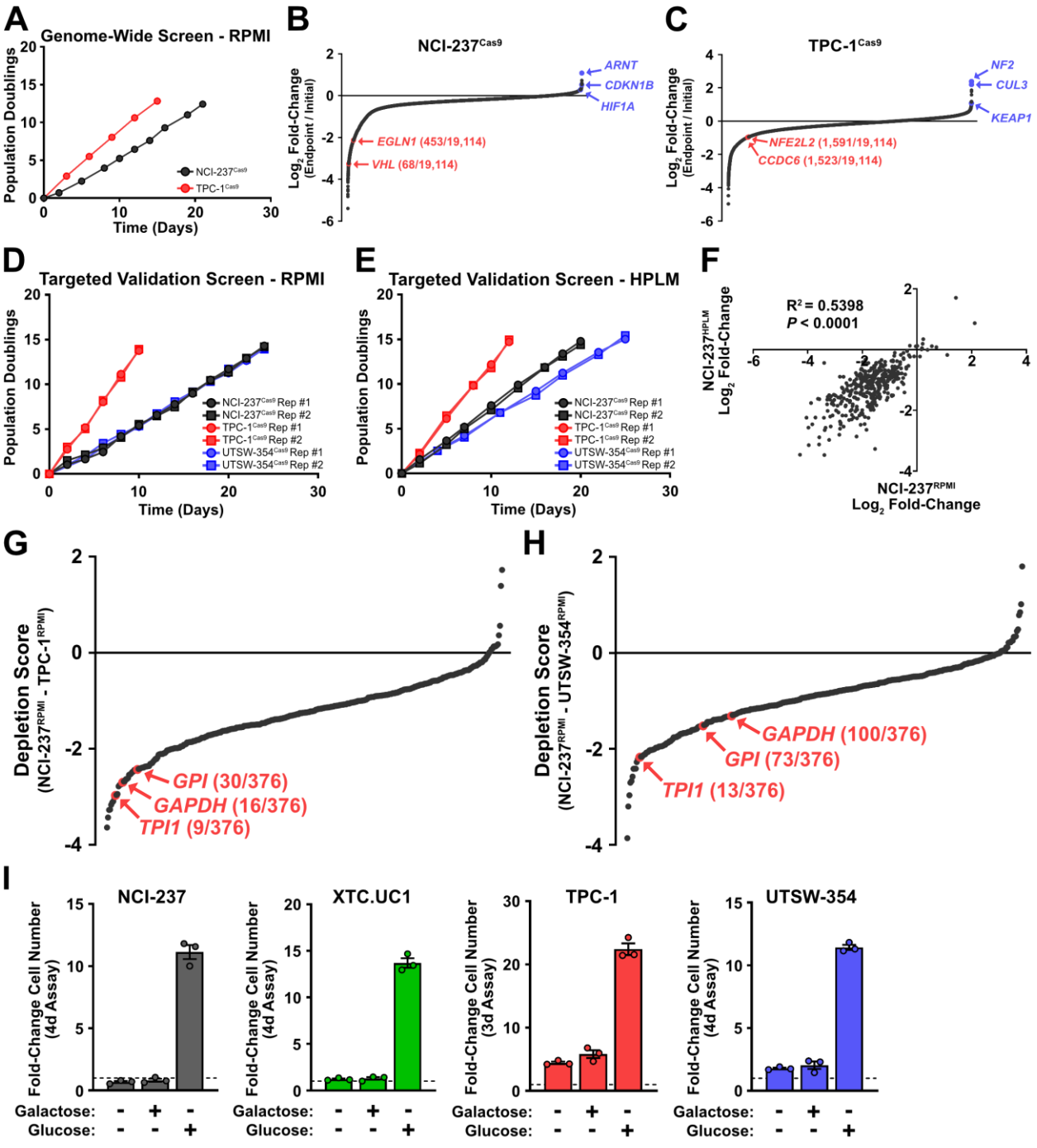
Supplementary Figure S1. A) Sanger sequencing trace of XTC.UC1 PCR-amplified *MT-ND1*. B) Tumor volume of NCI-237-R^{PDX} implanted subcutaneously in immunodeficient mice. Data are plotted as mean ± SD of 4 tumors. C) Sanger sequencing trace of *MT-ND5*. D) Sanger sequencing trace of *MT-ND1*. E) Sanger sequencing trace of *DNMT3A*. F) Sanger sequencing trace of *NF1*. G) Sanger sequencing trace of *NF2*. Sanger sequencing traces in C-G come from direct sequencing of PCR products amplified from NCI-237-$R^{PDX.}$ H) Tumor volume of NCI-237^{UTSW} cells injected subcutaneously in immunodeficient mice. Data are plotted as mean ± SD of 4 tumors. I) Sanger sequencing trace of *MT-ND5*. J) Sanger sequencing trace of *MT-ND1*. K) Sanger sequencing trace of *DNMT3A*. L) Sanger sequencing trace of *NF1*. M) Sanger sequencing trace of *NF2*. Sanger sequencing traces in I-M come from direct sequencing of PCR products amplified from NCI-237^{UTSW} cells. N) Oxygen consumption rates for intact cells; *n* = 6-8 replicates. Oligo = oligomycin; CCCP = carbonyl cyanide 3-chlorophenylhydrazone; Anti A = antimycin A. NCI-237^{xeno} cells are from an NCI-237^{UTSW} xenograft tumor formed in immunodeficient mice. Cells were assayed for oxygen consumption approximately 24 hours after tumor dissociation. Data are plotted as mean ± SEM. O) Schematic for labeling of key central carbon metabolites from [U-$^{13}C_6$] glucose under canonical oxidative conditions (*top*) or pyruvate carboxylase-mediated TCA cycle anaplerosis (*bottom*). PDH = pyruvate dehydrogenase; CS = citrate synthase; PCB = pyruvate carboxylase; GOT2 = glutamic-oxaloacetic transaminase 2; OAA = oxaloacetate. P) Mass isotopomer abundance for the indicated metabolites in cells cultured with [U-$^{13}C_6$] glucose for 6 hours. Data are plotted as mean ± SEM of 3 replicates. α-KG = alpha-ketoglutarate.

Supplementary Figure S1



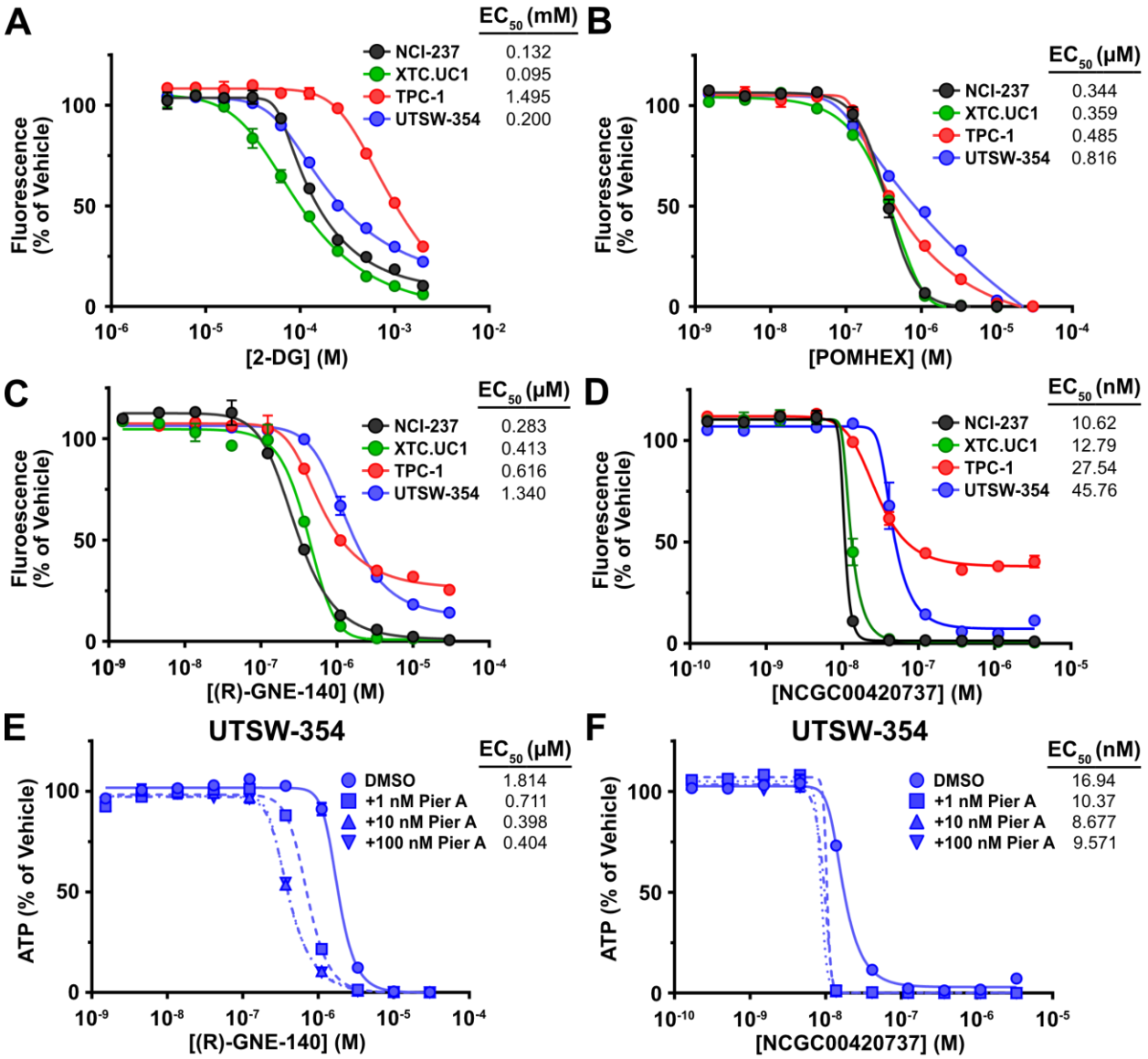
Supplementary Figure S2. A) Growth curves of NCI-237^{RPMI} and TPC-1^{RPMI} in primary genome-wide CRISPR-Cas9 knockout screen. B) Plot of genes for NCI-237^{RPMI} based on median log_2 fold-change in sgRNA abundance. C) Plot of genes for TPC-1^{RPMI} based on median log_2 fold-change in sgRNA abundance. Enriched genes are highlighted in blue; depleted genes are highlighted in red. D) Growth curves of NCI-237^{RPMI}, TPC-1^{RPMI}, and UTSW-354^{RPMI} replicates (*n* = 2) in targeted validation screen. E) Growth curves of NCI-237^{HPLM}, TPC-1^{HPLM}, and UTSW-354^{HPLM} replicates (*n* = 2) in targeted validation screen. F) Plot of gene log_2 fold-change values for NCI-237^{RPMI} and NCI-237^{HPLM} validation screens. Data reflect median log_2 fold-change in sgRNA abundance based on 2 replicates for each condition. G) Plot of genes from validation screen based on Depletion Score comparing NCI-237^{RPMI} and TPC-1^{RPMI}. H) Plot of genes from validation screen based on Depletion Score comparing NCI-237^{RPMI} and UTSW-354^{RPMI}. Depletion Score for G) and H) are calculated as [(NCI-237^{RPMI} gene log_2 fold-change) – (TPC-1^{RPMI}/UTSW-354^{RPMI} gene log_2 fold-change)]. Gene log_2 fold-change values reflect median log_2 fold-change in sgRNA abundance based on 2 replicates for each cell line. I) Fold-change in cell number of indicated cell lines in media lacking glucose or galactose, media containing 5 mM galactose, or media containing 5 mM glucose. Data are plotted as mean ± SEM of 3 replicates. Dashed line = 1.

Supplementary Figure S2



Supplementary Figure S3. A) Viability assay for cells treated with 2-DG for 3 days. B) Viability assay for cells treated with POMHEX for 3 days. C) Viability assay for cells treated with (R)-GNE-140 for 3 days. D) Viability assay for cells treated with NCGC00420737 for 3 days. E) Viability assay for UTSW-354 cotreated with (R)-GNE-140 and indicated doses of piericidin A for 3 days. F) Viability assay for UTSW-354 cotreated with NCGC00420737 and indicated doses of piericidin A for 3 days. Data are plotted as mean ± SEM of 2 replicates. G) Table of RNA-Seq FPKM values for *LDH* genes in indicated cell lines.

Supplementary Figure S3



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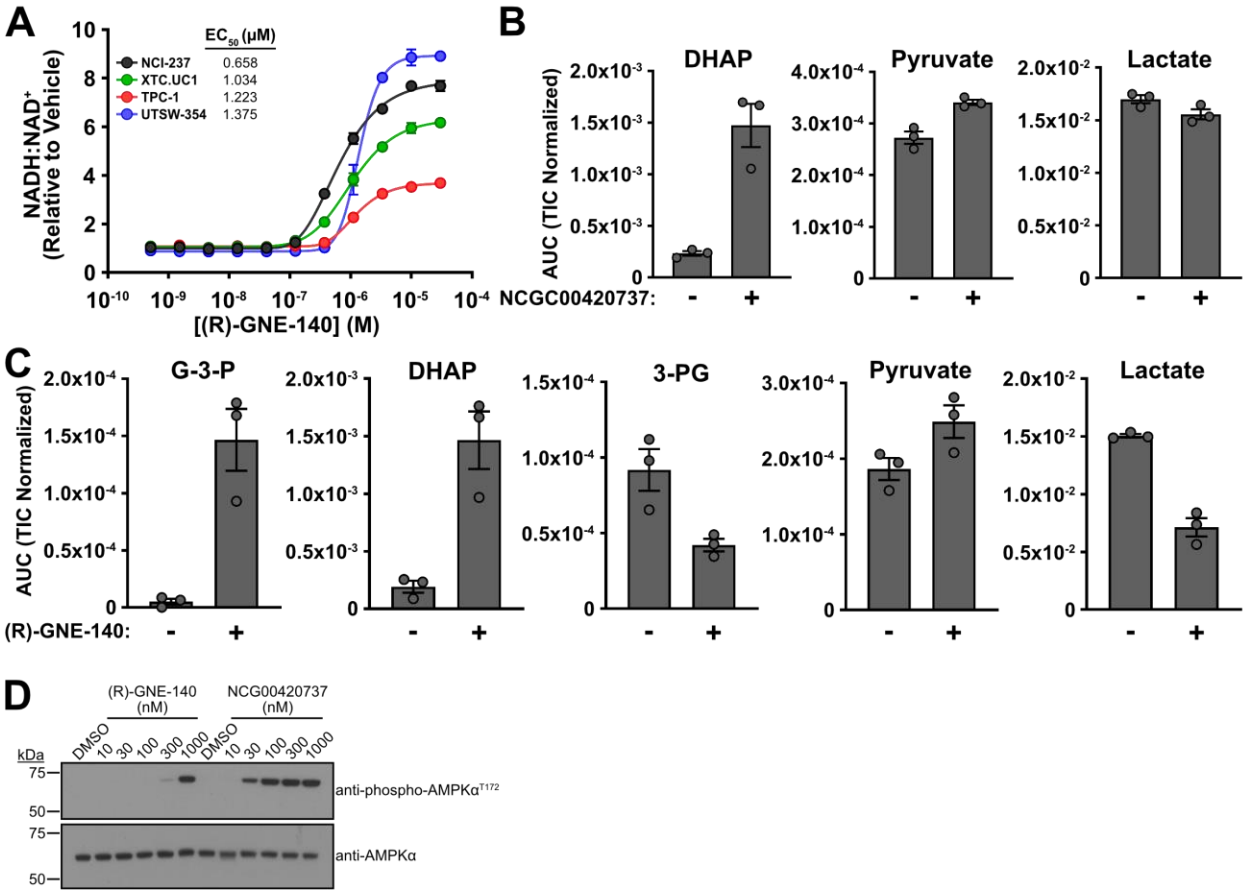
	RNASeq FPKM		
	NCI-237	TPC-1	UTSW-354
LDHA	778.65	819.35	765.55
LDHB	259.53	487.85	110.23
LDHC	0	0.2	0
LDHD	1.54	3.31	0.25

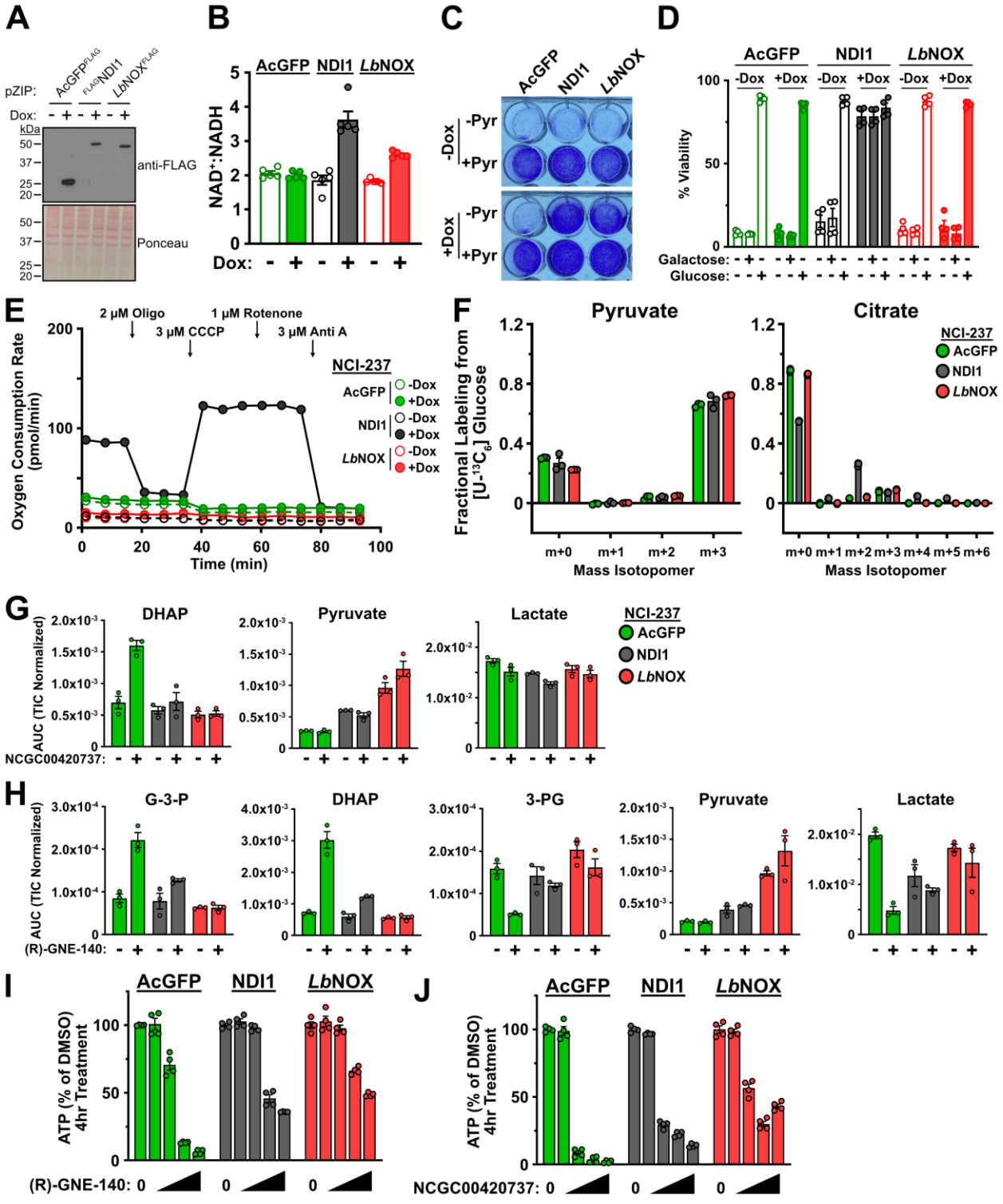
Supplementary Figure S4. A) NADH:NAD^+ ratio in cells treated with (R)-GNE-140 for 4 hours; *n* = 2 replicates. B) Metabolite levels in NCI-237^{UTSW} cells treated with 40 nM NCGC00420737 for 4 hours; *n* = 3 replicates. DHAP = dihydroxyacetone phosphate. C) Metabolite levels in NCI-237^{UTSW} cells treated with 1 μM (R)-GNE-140 for 4 hours; *n* = 3 replicates. G-3-P = glyceraldehyde-3-phosphate; DHAP = dihydroxyacetone phosphate; 3-PG = 3-phosphoglycerate. D) Immunoblot for phospho-AMPKα^{T172} and total AMPKα in NCI-237^{UTSW} cells treated with indicated doses of (R)-GNE-140 and NCGC00420737 for 4 hours.

Supplementary Figure S4



Supplementary Figure S5. A) Immunoblot for FLAG epitope tag in NCI-237^{UTSW} cells with inducible expression of indicated constructs. Cells were exposed to 100 ng/mL doxycycline for 24 hours prior to collection. B) NAD^+:NADH ratio in NCI-237^{UTSW} cells with inducible expression of indicated constructs. Cells were exposed to 100 ng/mL doxycycline for 24 hours prior to collection; *n* = 5 replicates. C) Crystal violet staining of NCI-237^{UTSW} cells cultured for 4 days under the indicated conditions. D) Viability of NCI-237^{UTSW} cells cultured under the indicated conditions for 48 hours; *n* = 4 replicates from 2 independent experiments. E) Oxygen consumption rates for intact NCI-237^{UTSW} cells expressing the indicated constructs; *n* = 6-8 replicates. Oligo = oligomycin; CCCP = carbonyl cyanide 3-chlorophenylhydrazone; Anti A = antimycin A. F) Mass isotopomer abundance for the indicated metabolites in NCI-237^{UTSW} cells cultured with [U-$^{13}C_6$] glucose for 4 hours; *n* = 3 replicates. G) Metabolite levels in NCI-237^{UTSW} cells expressing AcGFP, NDI1, or *Lb*NOX treated with 40 nM NCGC00420737 for 4 hours; *n* = 3 replicates. DHAP = dihydroxyacetone phosphate. H) Metabolite levels in NCI-237^{UTSW} cells expressing AcGFP, NDI1, or *Lb*NOX treated with 1 μM (R)-GNE-140 for 4 hours; *n* = 3 replicates. G-3-P = glyceraldehyde-3-phosphate; DHAP = dihydroxyacetone phosphate; 3-PG = 3-phosphoglycerate. I) ATP levels in cells treated with (R)-GNE-140 for 4 hours; *n* = 4 replicates from 2 independent experiments. Concentrations are 0.041, 0.37, 3.33, and 30 μM (R)-GNE-140. J) ATP levels in NCI-237^{UTSW} cells treated with NCGC00420737 for 4 hours; *n* = 4 replicates from 2 independent experiments. Concentrations are 0.014, 0.123, 1.11, and 10 μM NCGC00420737. Data for are plotted as mean ± SEM of indicated number of replicates.

Supplementary Figure S5



Supplementary Figure S6. A) Individual tumor volumes for NCI-237-R^{PDX} xenografts treated with vehicle (*n* = 5), 30 mg/kg NCGC00420737 (*n* = 4), or 60 mg/kg NCGC00420737 (*n* = 5). Vehicle or compound was administered once daily via jugular vein catheter for two treatment cycles (5 days on, 2 days off). A final compound administration was performed on Day 6 of Cycle 2 and animals were sacrificed 1 hour after receiving compound. B) Animal weight for NCI-237-R^{PDX}-bearing immunodeficient mice treated with vehicle (*n* = 5), 30 mg/kg NCGC00420737 (*n* = 4), or 60 mg/kg NCGC00420737 (*n* = 5). C) Red blood cell (left), hematocrit (center), and hemoglobin (right) levels in EDTA-treated whole blood. Blood was collected 1 hour after final administration of compound. D) Volcano plot comparing metabolite levels in NCI-237-R^{PDX} tumors treated with vehicle or 30 mg/kg NCGC00420737. Metabolite levels were determined using LC-MS/MS. Horizontal dashed line = 1.3 (*P*-value ≅ 0.05); vertical dashed lines = -1 and 1. E) Volcano plot comparing metabolite levels in NCI-237-R^{PDX} tumors treated with vehicle or 60 mg/kg NCGC00420737. Metabolite levels were determined using LC-MS/MS. Horizontal dashed line = 1.3 (*P*-value ≅ 0.05); vertical dashed lines = -1 and 1. *P*-values in D) and E) were determined using two-sided *T*-test assuming unequal variance. F) Immunoblot for phospho-AMPKα^{T172} and total AMPKα in NCI-237-R^{PDX} tumors. G) Plasma and tumor concentrations of NCGC00420737 determined by LC-MS/MS. Blood and tumors were collected 1 hour after final administration of compound. Data are plotted as mean ± SD of indicated number of replicates. H) Individual tumor volumes for NCI-237-R^{PDX} xenografts treated with vehicle (*n* = 5) or 60 mg/kg NCGC00420737 (*n* = 5). Vehicle or compound was administered once daily via jugular vein catheter for the indicated number of treatment cycles (5 days on, 2 days off). Three animals receiving 60 mg/kg NCGC00420737 died between the end of Cycle 3 and start of Cycle 4. A final compound administration was performed on Day 2 of Cycle 4 and animals were sacrificed 1 hour after receiving compound. I) Final tumor mass for NCI-237-R^{PDX} xenografts from Fig. 5G. J) Animal weight for NCI-237-R^{PDX}-bearing immunodeficient mice treated with vehicle (*n* = 5) or 60 mg/kg NCGC00420737 (*n* = 5). K) Red blood cell and hematocrit levels in EDTA-treated whole blood. Blood was collected 1 hour after final administration of compound.

Supplementary Figure S6