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Supplemental information

KEAP1 deficiency drives glucose dependency and sensitizes lung cancer cells and tumors to GLUT inhibition

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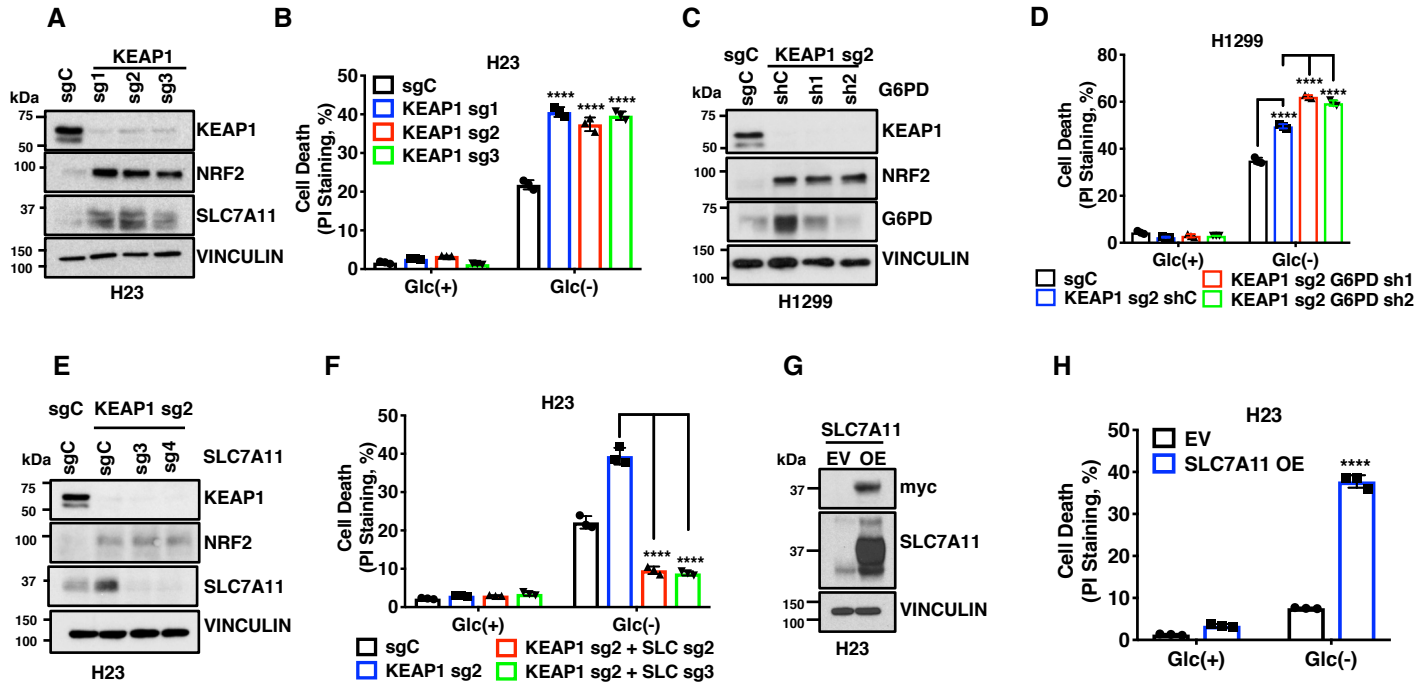
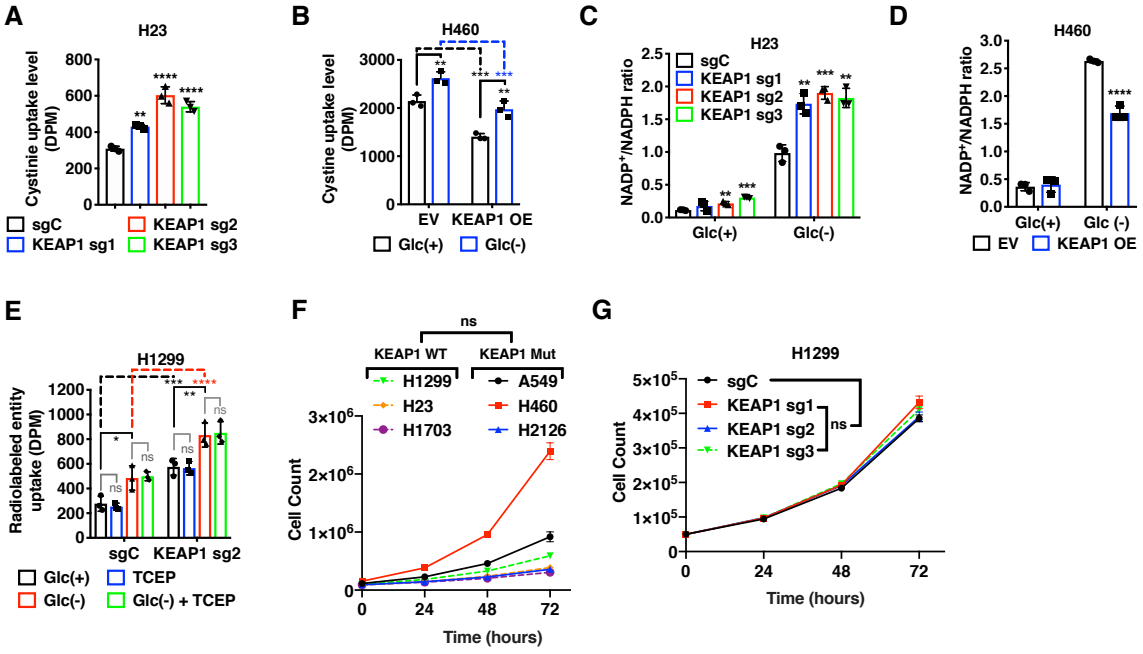
Supplementary Figure 1, Related to Figure 1



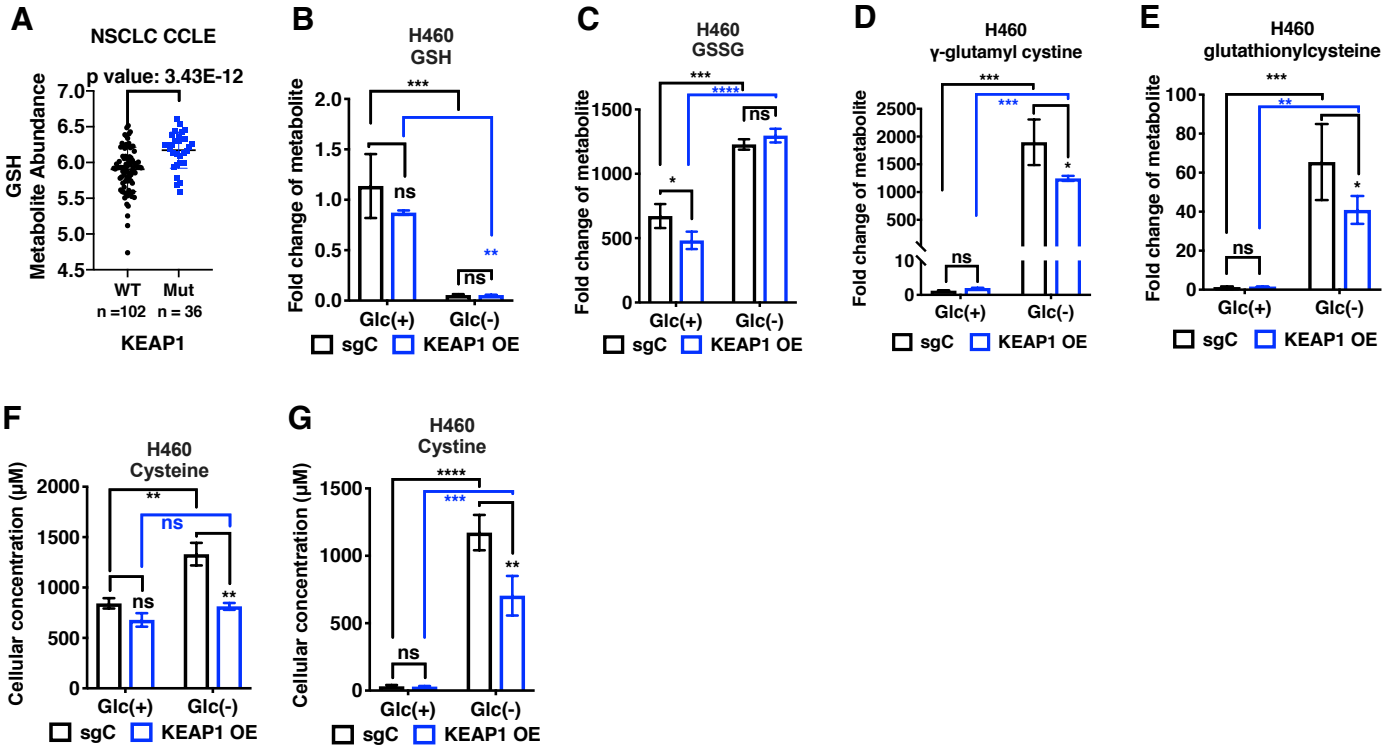
Supplementary Figure 1. KEAP1 deficiency promotes glucose dependency in lung cancer cells mainly through SLC7A11. A, Western blotting analysis of KEAP1, NRF2, SLC7A11 protein levels in the control (sgC) and KEAP1 knockout (sg1/2/3) H23 cells. Vinculin is used as a loading control. B, Cell death in KEAP1 knockout H23 cells upon glucose withdrawal was analyzed by PI staining. C, Western blotting analysis of KEAP1, NRF2, G6PD protein levels in G6PD knockdown in KEAP1 knockout H1299 cells. D, Cell death upon glucose withdrawal was analyzed by PI staining in KEAP1 knockout H1299 cells with G6PD knocked down. E, Western blotting analysis of KEAP1, NRF2, SLC7A11 protein levels in SLC7A11-KEAP1 double knockout H23 cells. F, Cell death in SLC7A11-KEAP1 double knockout H23 cells upon glucose withdrawal was analyzed by PI staining. G, SLC7A11 protein levels in SLC7A11-overexpressing H23 cells. EV, empty vector, OE, SLC7A11 overexpressing. H, cell death in SLC7A11-overexpressing H123 cells upon glucose withdrawal was analyzed by PI staining. Data are represented as mean +/- SD; n=3. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$

Supplementary Figure 2, Related to Figure 2



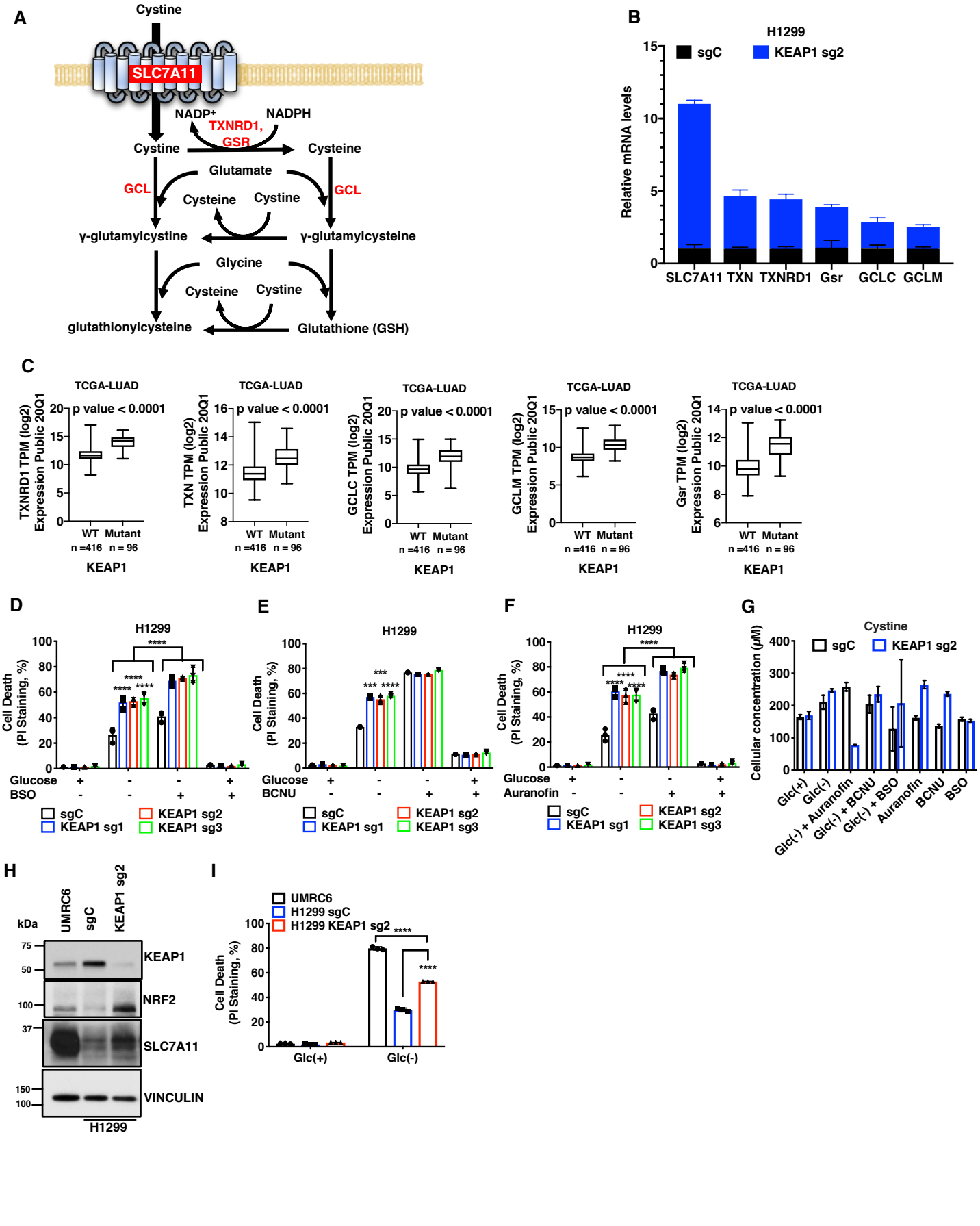
Supplementary Figure 2. **Glucose dependency of KEAP1 deficient cells is partly due to SLC7A11 mediated increased cystine uptake mediated NADPH consumption.** A, Cystine uptake levels in KEAP1 knockout H23 cells upon control (Glc(+)) and glucose starvation (Glc(-)) conditions. B, Cystine uptake levels in KEAP1 overexpressing H460 cells upon control (Glc(+)) and glucose starvation (Glc(-)) conditions. C, NADP+/NADPH KEAP1 knockout H23 cells cultured with glucose (Glc(+)) or without glucose (Glc(-)) for 24 h. D, NADP+/NADPH ratios in control and KEAP1 overexpressing H460 cell lines upon glucose starvation. E, Cystine uptake levels in different KEAP1 knockout H1299 cells treated with glucose starvation and TCEP supplementation. F, Cell proliferation rates of NSCLC cell lines carrying WT KEAP1 (H1299, H23, H1703) or mut KEAP1 (A549, H460, H2126). G, Cell proliferation rates of KEAP1 knockout H1299 cells. Data are represented as mean +/- SD; n=3. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Figure 3, Related to Figure 3



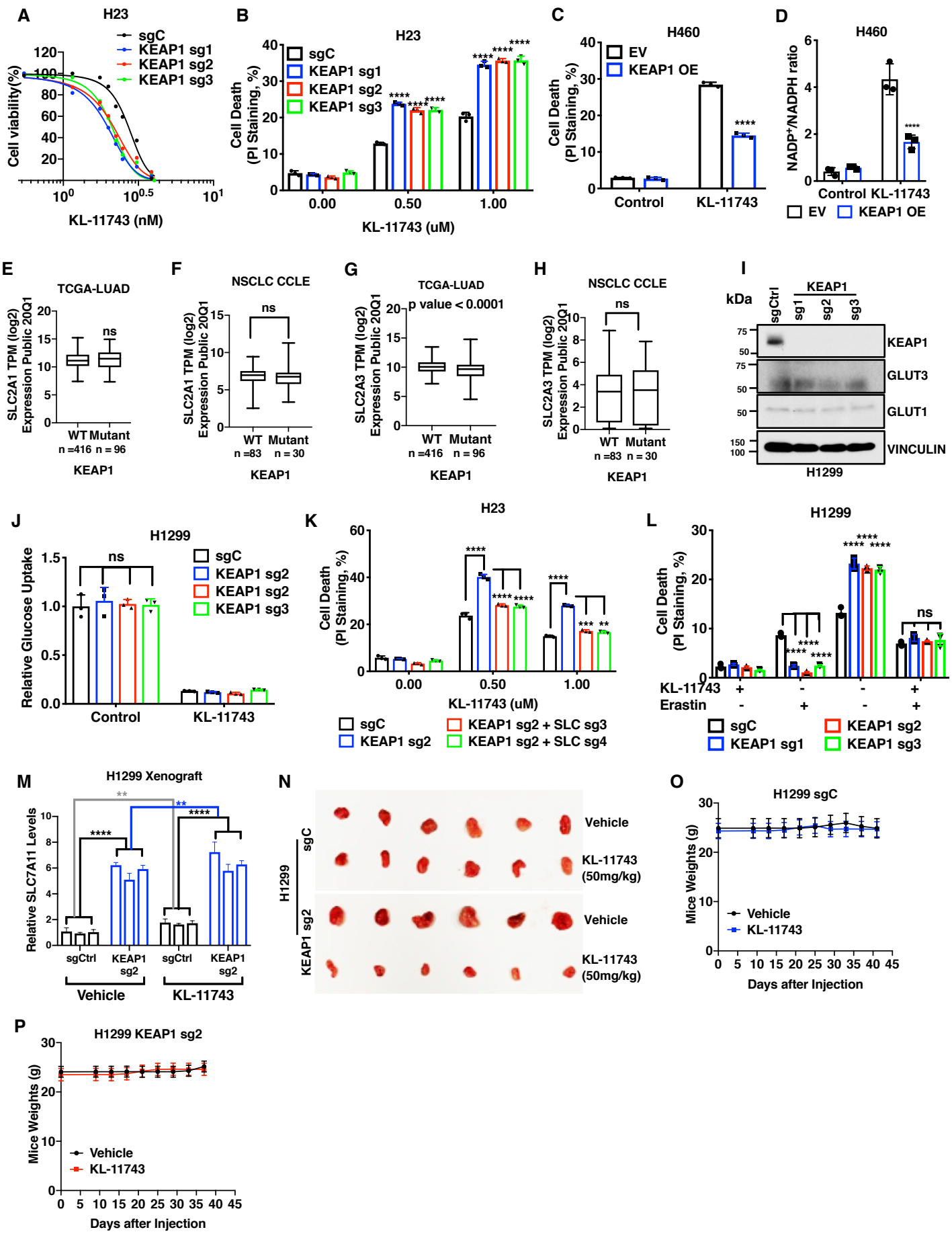
Supplementary Figure 3. KEAP1 deletion in lung cancer cells leads to buildup of disulfide molecules upon glucose starvation. A, Correlation analysis of glutathione (GSH) metabolite abundance with KEAP1 mutation status of NSCLC cell lines in the CCLE database. B-G, The concentrations or relative fold changes of glutathione (GSH; B), oxidized glutathione (GSSG; C), γ-glutamylcystine (D), glutathionylcysteine (E), Cysteine (F), and Cystine (G) in *KEAP1* overexpressing H460 cells upon glucose starvation. Data are represented as mean +/- SD; n=3. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Figure 4, Related to Figure 3



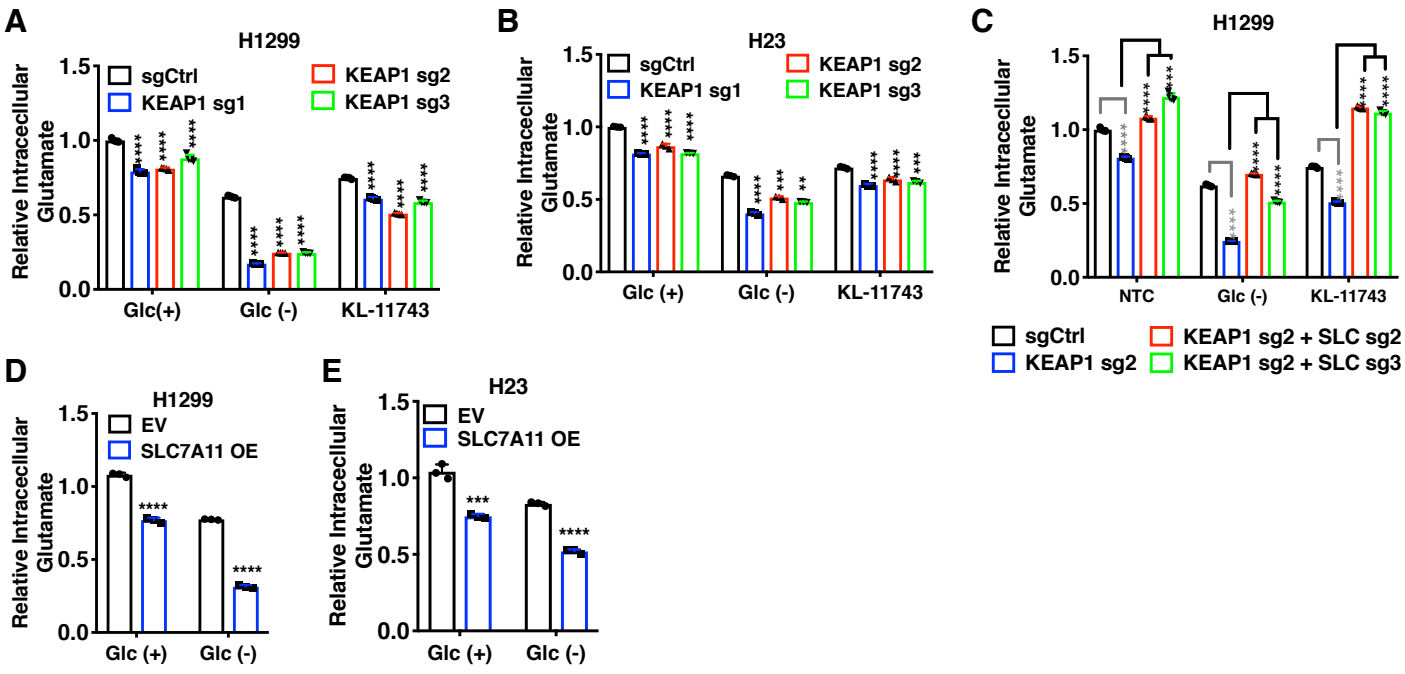
Supplementary Figure 4. KEAP1-NRF2 axis supports disulfide buildup upon glucose starvation. A, Schematic representation of disulfide stress pathways in KEAP1 KO cells regulated by NRF2 target genes including glutamate-cysteine ligase (GCL, the rate-limiting enzyme in GSH biosynthesis which consists of GCLM and GCLC), glutathione reductase (GSR), and thioredoxin reductase 1 (TXNRD1). B, qPCR quantification of indicated NRF2 target genes in the KEAP1 KO H1299 cells. C, Correlation analysis of indicated NRF2 target genes with KEAP1 mutation status of LUAD tumors from TCGA. Cell death analysis of H1299 KEAP1 knockout cells cultured in complete medium or glucose-free medium supplemented with buthionine sulfoximine (BSO) (D), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (E), Auranofin (F) for 24 h. G, Cystine accumulation in KEAP1 KO H1299 cells treated with or without glucose along with indicated supplements for 8 hrs. H, Western blotting analysis of KEAP1, NRF2, SLC7A11 protein levels in UMRC6, H1299 (sgC) and KEAP1 knockout (sg2) cells. Vinculin is used as a loading control. I, Cell death analysis of UMRC6, H1299 (sgC) and KEAP1 knockout (sg2) cells upon glucose starvation for 24 hrs. Data are represented as mean +/- SD; n=3. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Figure 5, Related to Figure 4



Supplementary Figure 5. KEAP1 deficiency sensitizes lung cancer cells or lung tumors to GLUT inhibition A, Cell viability in KEAP1 KO H23 cell lines upon GLUT inhibitor (KL-11743) treatment was analyzed by CCK8 (A). Cell death in KEAP1 KO H1299 cells upon GLUT inhibitor treatment was measured by PI staining (B). C, Cell viability of KEAP1 overexpressing H460 cells treated with KL-11743. D, NADP+/NADPH ratios in control and KEAP1 overexpressing H460 cell lines upon KL-11743 treatment. Correlation analysis of SLC2A1 expression in KEAP1 WT vs mutant LUAD tumors from TCGA (E) and NSCLC cell lines in CCLE database (F). Correlation analysis of SLC2A3 expression in KEAP1 WT vs mutant LUAD tumors from TCGA (G) and NSCLC cell lines in CCLE database (H). I, Western blot analysis of GLUT1, GLUT3, KEAP1 protein expression in KEAP1 KO H1299 cells. Vinculin was used as loading control. J, Glucose uptake in KEAP1 KO H1299 cells treated with 1 μM KL-11743. DPM, disintegrations per min. K, Cell death in SLC7A11-KEAP1 double KO H23 cell lines upon GLUT inhibitor (KL-11743) treatment was analyzed by PI staining. L, Cell death analysis of H1299 KEAP1 knockout cells cotreated with erastin and KL-11743. M, qPCR quantification of SLC7A11 levels in indicated xenograft tumors derived from H1299 treated with vehicle or KL-11743. N, O, P, Mice weights of H1299 Xenografts derived from sgC (M) or KEAP1 sg2 (N) at different time points treated with vehicle or KL-11743. Data are represented as mean +/- SD; n=3. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Figure 6



Supplementary Figure 6. KEAP1 deficiency drives glutamate export through SLC7A11 Intracellular glutamate levels were determined after 6h of glucose (Glc) withdrawal in (A) KEAP1 knockout H1299, (B) KEAP1 knockout H23, (C) KEAP1 SLC7A11 double knockout H1299, SLC7A11 overexpressing (D) H1299 and (E) H23 cells. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Table 1, Related to Figure 1

	H2126	H460	A549	H1299	H23	H1703
KEAP1	Mut	Mut	Mut	WT	WT	WT
TP53	Mut	WT	WT	WT	Mut	Mut
KRAS	WT	Mut	Mut	WT	Mut	WT
LKB1	WT	Mut	Mut	WT	Mut	WT
PIK3CA	WT	Mut	WT	WT	WT	WT

Table S1. KEAP1 mutation status of NSCLC cell lines Mutation status of lung cancer genes in the lung cancer cell lines used in this study.

Supplementary Table 2, Related to Figure 1

KEAP1 WT						KEAP1 Mut	
SLC7A11 log2(TPM+1) Expression Public 20Q1	Cell Line Name	SLC7A11 log2(TPM+1) Expression Public 20Q1	Cell Line Name	SLC7A11 log2(TPM+1) Expression Public 20Q1	Cell Line Name	SLC7A11 log2(TPM+1) Expression Public 20Q1	Cell Line Name
0.54596837	CORL23	2.96532255	DV90	4.30815698	NCIH292	2.207892852	NCIH661
0.63226822	HCC2429	3.00360224	A427	4.31831684	NCIH2291	2.454175893	NCIH23
0.90303827	NCIH2882	3.04264434	CORL105	4.32696871	RERFLCSQ1	2.867896464	HCC2108
0.91073266	NCIH2342	3.0583165	HCC2279	4.37573454	LOUNH91	3.360364277	HCC2935
1.38404981	IALM	3.06522762	HCC1195	4.42961596	SKLU1	3.379898164	NCIH838
1.45417589	NCIH1666	3.06695024	HCC4006	4.45088132	NCIH1437	3.641546029	NCIH1339
1.46988598	NCIH810	3.11769504	NCIH1563	4.52606881	NCIH2405	4.181897643	NCIH1915
1.51096192	NCIH2085	3.14077866	CALU6	4.6363346	NCIH2087	4.297191417	NCIH1435
1.65076456	NCIH1963	3.169925	ABC1	4.70873904	CALU3	4.510329019	BEN
1.70043972	NCIH526	3.22958792	NCIH1869	4.79960542	NCIH1703	4.736063628	MORCPR
1.70043972	HOP92	3.23419472	NCIH1650	4.88508623	RERFLCKJ	4.809414444	NCIH2030
1.85199884	COLO699	3.23572706	NCIH2009	4.99322147	NCIH1651	5.21023299	NCIH2172
1.87578006	NCIH650	3.24640809	HCC461	5.14159628	NCIH441	5.274261661	NCIH1648
1.91073266	HCC515	3.28983447	NCIH2347	5.2156786	LCLC97TM1	5.321567376	NCIH2126
1.95233357	NCIH2106	3.36457243	SW900	5.29939121	RERFLCAI	5.34091827	LUDLU1
1.96347412	NCIH1975	3.36737107	RERFLCAD1	5.47961861	HCC2814	5.514753498	NCIH460
2.00360224	HCC364	3.37573454	NCIH1155	5.49441561	NCIH854	5.756489607	NCIH2170
2.08065766	HCC827	3.42492209	LU99	5.6129422	KNS62	5.818134761	HCC44
2.12432814	SW1573	3.45680615	NCIH2444	5.71918344	NCIH2887	5.902556005	NCIH1385
2.14077866	NCIH1299	3.51222689	HCC1359	5.73470962	PC14	5.904724928	NCIH2110
2.23878686	NCIH522	3.53977919	NCIH226	5.73660488	EBC1	5.946496941	NCIH1355
2.26002566	HCC78	3.63691458	CAL12T	5.77741972	HOP62	5.948367232	NCIH1623
2.38128337	HCC827GR5	3.63807384	NCIH358	5.85574059	LK2	5.9800253	NCIH2023
2.40326772	NCIH2073	3.66334462	PC9	5.98891186	NCIH520	6.000225403	HCC15
2.44625623	NCIH1693	3.7059779	NCIH1781	6.02635745	LC1F	6.02103548	T3M10
2.46205232	NCIH1092	3.74523733	HCC1171	6.09296851	HCC95	6.04176865	NCIH322
2.49825087	NCIH1581	3.87282876	NCIH1734	6.10873353	HCC1438	6.102868054	A549
2.54349588	RERFLCAD2	4.02236781	LCLC103H	6.38973888	NCIH1373	6.167719199	NCIH1944
2.68706069	NCIH1568	4.05311134	LU65	6.4849437	NCIH1793	6.459103696	RERFLCMS
2.75915583	HCC2450	4.09423607	NCIH3122	6.50080205	HARA	6.736063628	NCIH1755
2.78659636	SQ1	4.138323	NCIH1838	6.74779018	SKMES1	6.85785662	NCIH1792
2.82984956	EPLC272H	4.14404637	NCIH596	7.06501215	NCIH2228	6.871597266	NCIH1573
2.85399565	NCIH3255	4.21023299	CALU1			6.891176125	NCIH2122
2.94110631	EKVX	4.26153082	HCC366			7.221103725	LXF289
2.96532255	NCIH1395	4.28835856	CHAGOK1			7.379291716	NCIH647

Table S2. SLC7A11 has a positive correlation with KEAP1 mutation in NSCLC cell lines SLC7A11 expression in KEAP1 WT and mutant NSCLC cell lines available on CCLE database.

Supplementary Table 3, Related to Figure 4

Sr. No.	PDX ID	Sensitivity to KL-11743	SLC7A11 expression	KEAP1	TP53	KRAS	STK11/LKB1	PIK3CA	NFE2L2
1	TC393	insensitive	58	WT	WT	WT	WT	WT	WT
2	TC551	insensitive	70	WT	WT	G12C	WT	WT	WT
3	**TC333**	**sensitive**	10876	R601W	E273H	G12D	WT	WT	WT
4	**TC494**	**sensitive**	9139	N68S	WT	G12V	Splicing mut	WT	WT
5	**TC453**	**sensitive**	2869	WT	WT	G12C	Splicing mut	WT	WT

Table S3. *KEAP1* mutant PDX derived tumors are sensitive to GLUT inhibitor Mutation status of lung cancer genes in the PDX models used in our previous study (Liu et al. 2020).

Supplementary Table 4

Sr. No.	Primer sequence	Gene
1	ATGCAGTGGCAGTGACCTTT	SLC7A11-F
2	GGCAACAAAGATCGGAACTG	SLC7A11-R
3	TTTCAGGAAGCCTTGGACGCT	TXN-F
4	GCAACATCCTGACAGTCATCCAC	TXN-R
5	ACGGTGATGCTGGCAATAGG	TXNRD-F
6	CTGGGGTGAGCTCCACCTTA	TXNRD-R
7	ACCCCGATGTATCACGCAGTTA	Gsr-F
8	TGTCAAAGTCTGCCTTCGTTGC	Gsr-R
9	GGCACAAGGACGTTCTCAAGT	GCLC-F
10	CAAAGGGTAGGATGGTTTGGG	GCLC-R
11	CTCATTCCGCTGTCCAGGT	GCLM-F
12	CCTTTGCAGATGTCTTTCCTGAA	GCLM-R
13	CGGAACCGCTCATTGCC	b-Actin-F
14	ACCCACACTGTGCCCATCTA	b-Actin-R

Table S4. Primer sequences RT-PCR primer sequences used in this study.