Supplementary Information

A targetable CoQ-FSP1 axis drives ferroptosis- and radiation-resistance in KEAP1 inactive lung cancers

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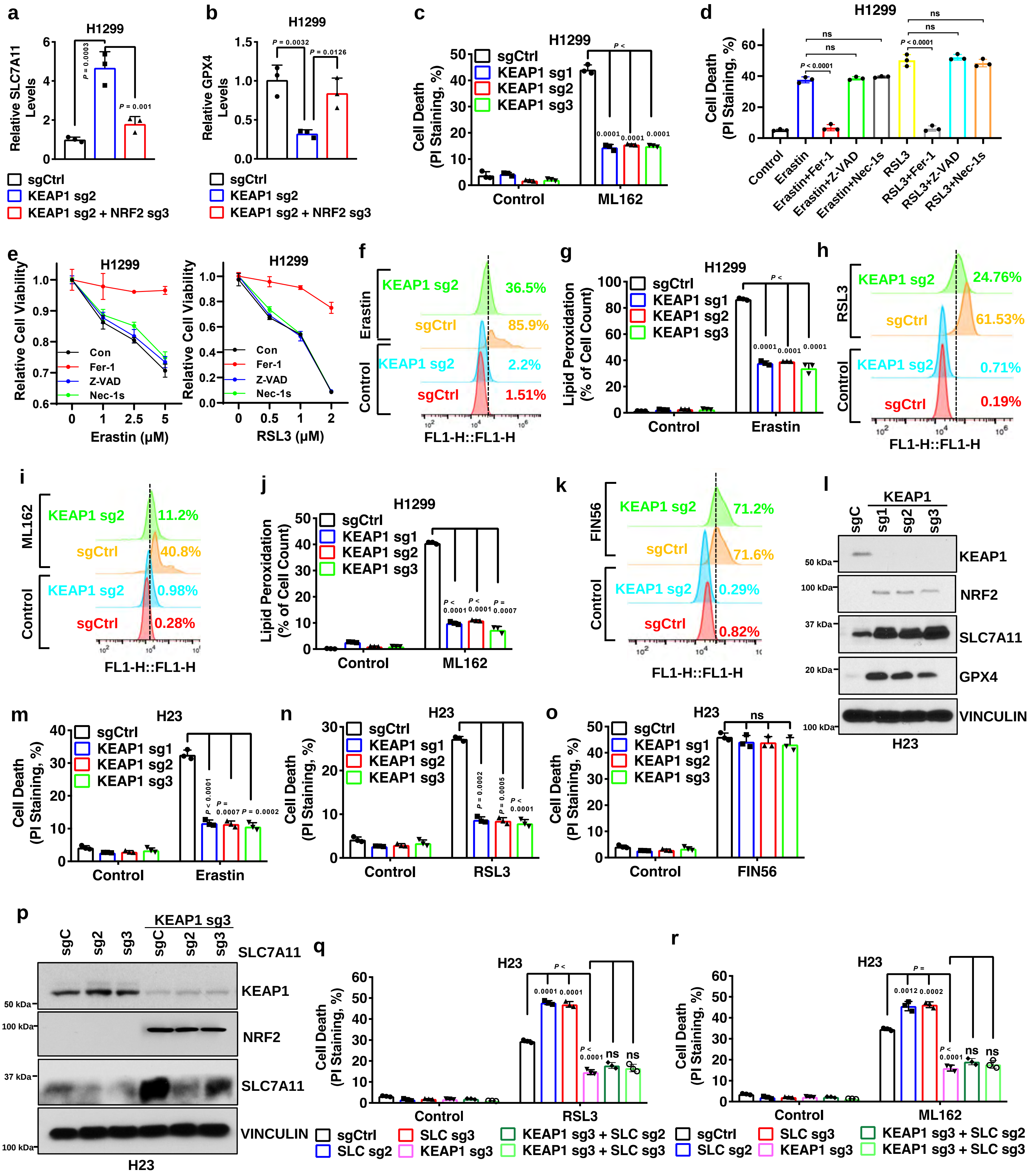
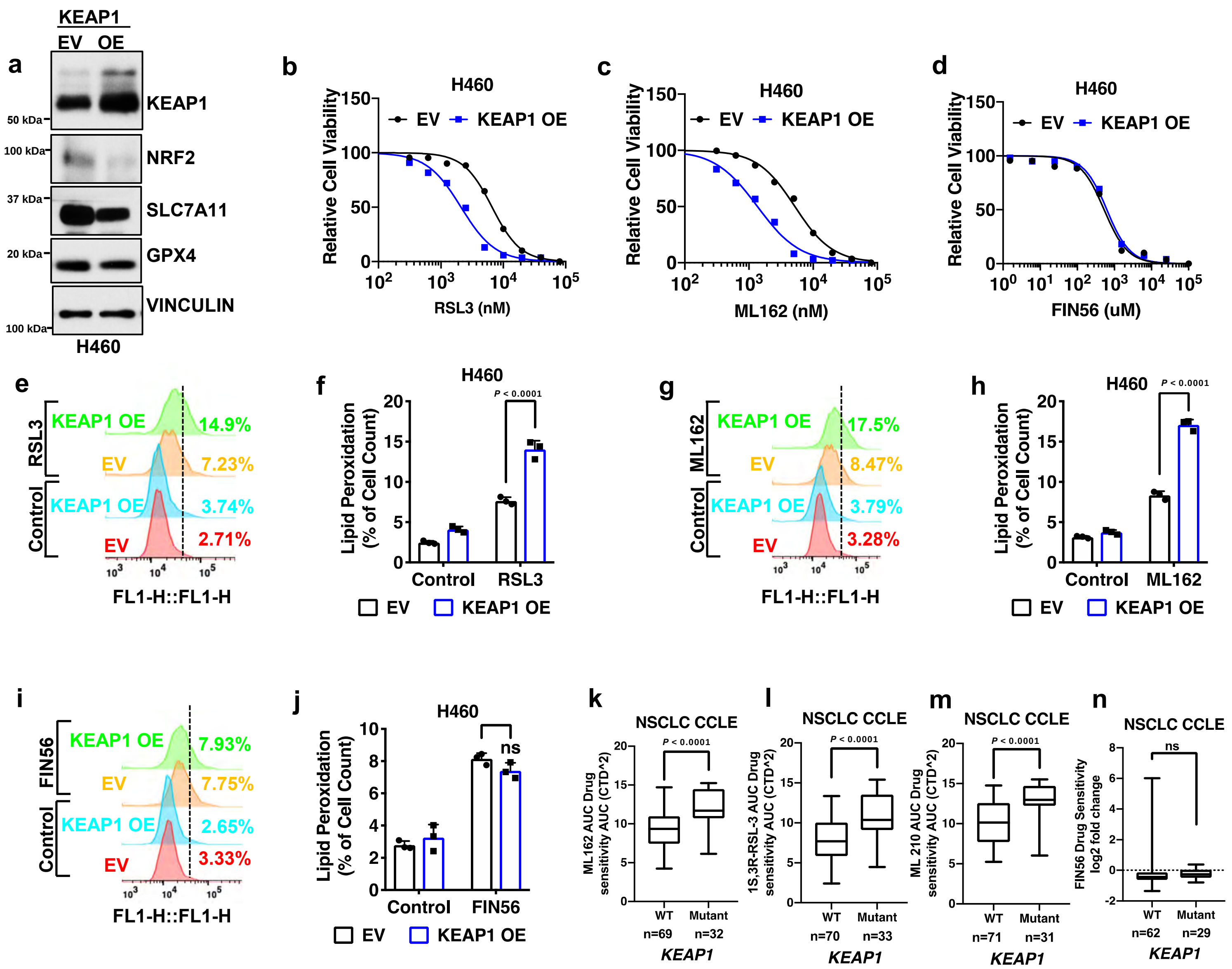
Supplementary Figure 1



Supplementary Figure 1. KEAP1 regulates ferroptosis in a SLC7A11-independent manner in lung cancer cells.

a, b qRT-PCR analysis of *SLC7A11* (**a**) and *GPX4* (**b**) in *KEAP1/ NRF2* DKO H1299 cells. **c** Cell death upon ML162 treatment in *KEAP1* KO H1299 cells was analyzed by PI staining. **d, e** Cell death (**d**) and cell viability (**e**) measurement in *KEAP1* KO H1299 cells with indicated treatments. **f-k** Lipid peroxidation levels were determined for *KEAP1* KO H1299 cells treated with erastin (**f, g**), RSL3 (**h**), ML162, (**i, j**) and FIN56 (**k**). **l** Protein levels of KEAP1, NRF2, SLC7A11, GPX4 in *KEAP1* KO H23 cells were determined by western blotting. **m-o** Cell death upon erastin (**m**), RSL3 (**n**), or FIN56 (**o**) treatment in *KEAP1* KO H23 cells was analyzed by PI staining. **p** Protein levels of KEAP1, NRF2, SLC7A11 in *SLC7A11* KO, *KEAP1* KO and *SLC7A11/ KEAP1* DKO H23 cells. **q, r** Cell death was quantified by PI staining for H23 cells by RSL3 (**q**) or ML162 (**r**). Error bars are (if mentioned otherwise) mean +/- SD; *n* =3. P value was determined by two-tailed unpaired Student's t test (1a, b, d) and 2-way ANOVA (1c, 1g, 1j, 1m-o, 1q-r); ns, not significant. Source data are provided as a Source Data file.

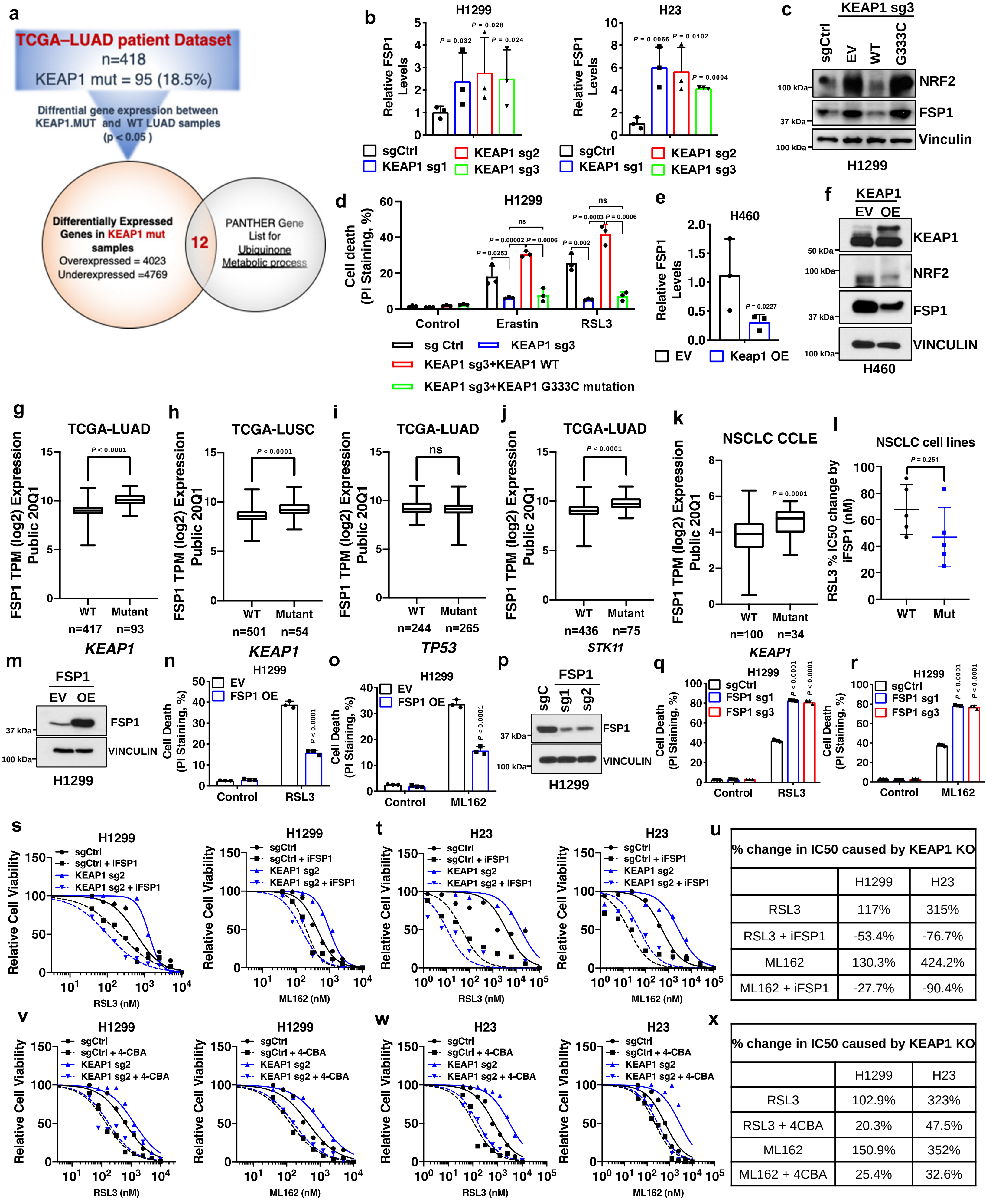
Supplementary Figure 2



Supplementary Figure 2. KEAP1 regulates ferroptosis in lung cancer cells.

a Protein levels of KEAP1, NRF2, SLC7A11, GPX4 in H460 cells with KEAP1 overexpression (OE). **b-d** Cell viability was quantified by CCK8 for H460 cells by RSL3 (**b**), ML162 (**c**) and FIN56 (**d**). **e-j** Lipid peroxidation levels were determined for KEAP1 OE H460 cells treated with RSL3 (**e, f**), ML162 (**g, h**), or FIN56 (**i, j**). **k-n** *KEAP1* mutation correlates with resistance to ML162 (**k**), RSL3 (**l**), and ML210 (**m**), but not FIN56 (**n**). NSCLC: Non-Small Cell Lung Cancer; CCLE: Cancer Cell Line Encyclopedia. Error bars are (if mentioned otherwise) mean +/- SD; *n* =3. P value was determined by two-tailed unpaired Student's t test. Box and whisker plots show centre line at median, box limits at 25th/75th centiles and whiskers ±1.5× interquartile range (IQR); ns, not significant. Source data are provided as a Source Data file.

Supplementary Figure 3



u

% change in IC50 caused by KEAP1 KO		
	H1299	H23
RSL3	117%	315%
RSL3 + iFSP1	-53.4%	-76.7%
ML162	130.3%	424.2%
ML162 + iFSP1	-27.7%	-90.4%

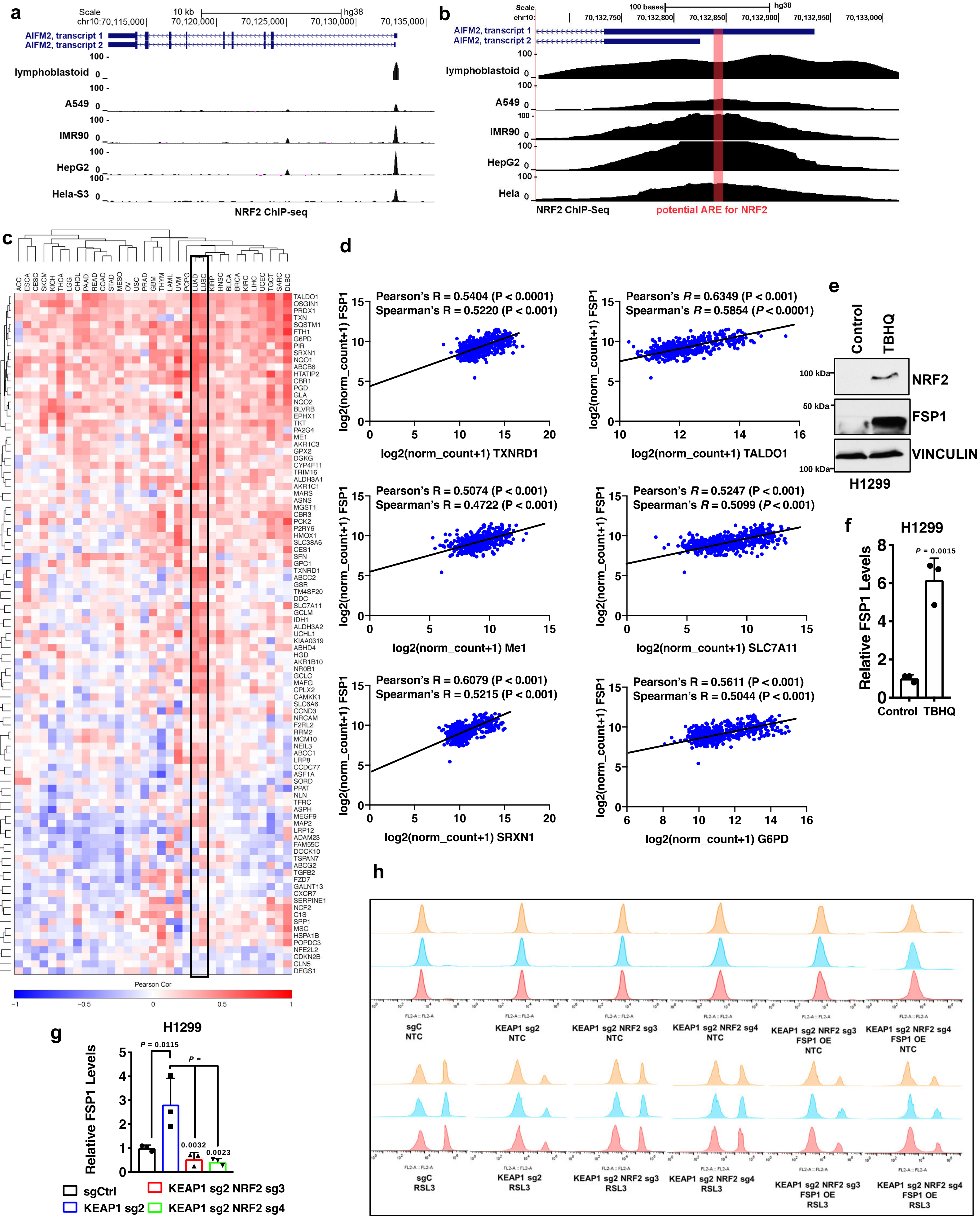
x

% change in IC50 caused by KEAP1 KO		
	H1299	H23
RSL3	102.9%	323%
RSL3 + 4CBA	20.3%	47.5%
ML162	150.9%	352%
ML162 + 4CBA	25.4%	32.6%

Supplementary Figure 3. KEAP1 regulates ferroptosis sensitivity through FSP1.

a Schematic of gene ontology analysis on overexpressed genes in *KEAP1* mutant LUAD. **b** qRT-PCR analysis of *FSP1* in *KEAP1* KO H1299 or H23 cells. **c** Protein levels of NRF2 and FSP1 in H1299 control cells or in H1299 *KEAP1* KO cells expressing empty vector (EV), KEAP1 wild-type (WT), or KEAP1 G333C mutant. **d** Cell death analysis of indicated H1299 cells treated with erastin or RSL3. **e** qRT-PCR analysis of *FSP1* in H460 cells with KEAP1 overexpression (OE). **f** Protein levels of KEAP1, NRF2, and FSP1 in KEAP1 OE H460 cells. **g-k** Correlation analysis of FSP1 expression levels in *KEAP1* mutant TCGA-LUAD (**g**), *KEAP1* mutant TCGA-LUSC (**h**), *p53* mutant TCGA-LUAD (**i**), *LKBI* mutant TCGA-LUAD (**j**), or *KEAP1* mutant NSCLC cell lines from CCLE datasets (**k**). TCGA: The Cancer Genome Atlas. **l** Percentage changes in RSL3 IC50 with iFSP1 cotreatment in NSCLC cell lines. **m** protein levels of FSP1 in H1299 cells with FSP1 OE. **n, o** Cell death analysis of H1299 cells with FSP1 OE treated with RSL3 (**n**) and ML162 (**o**). **p** Protein levels of FSP1 in *FSP1* KO H1299 cells. **q, r** Cell death analysis of *FSP1* KO H1299 cells treated with RSL3 (**q**) or ML162 (**r**). **s, t** Cell viability was quantified by CCK8 in *KEAP1* KO H1299 cells with iFSP1 treatment combined with RSL3 or ML162 (**s**); *KEAP1* KO H23 cells with iFSP1 treatment combined with RSL3 or ML162 (**t**). **u** Percentage changes in IC50 caused by *KEAP1* KO in H1299 and H23 cell lines upon the indicated treatments. **v, w** Cell viability was quantified by CCK8 in *KEAP1* KO H1299 cells with 4-CBA treatment combined with RSL3 or ML162 (**v**); *KEAP1* KO H23 cells with 4-CBA treatment combined with RSL3 or ML162 (**w**). **x** Percentage changes in IC50 caused by *KEAP1* KO in H1299 and H23 cell lines upon the indicated treatments. Error bars are (if mentioned otherwise) mean +/- SD; *n* =3. P value was determined by two-tailed unpaired Student's t test. Box and whisker plots show centre line at median, box limits at 25th/75th centiles and whiskers ±1.5× interquartile range (IQR); ns, not significant. Source data are provided as a Source Data file.

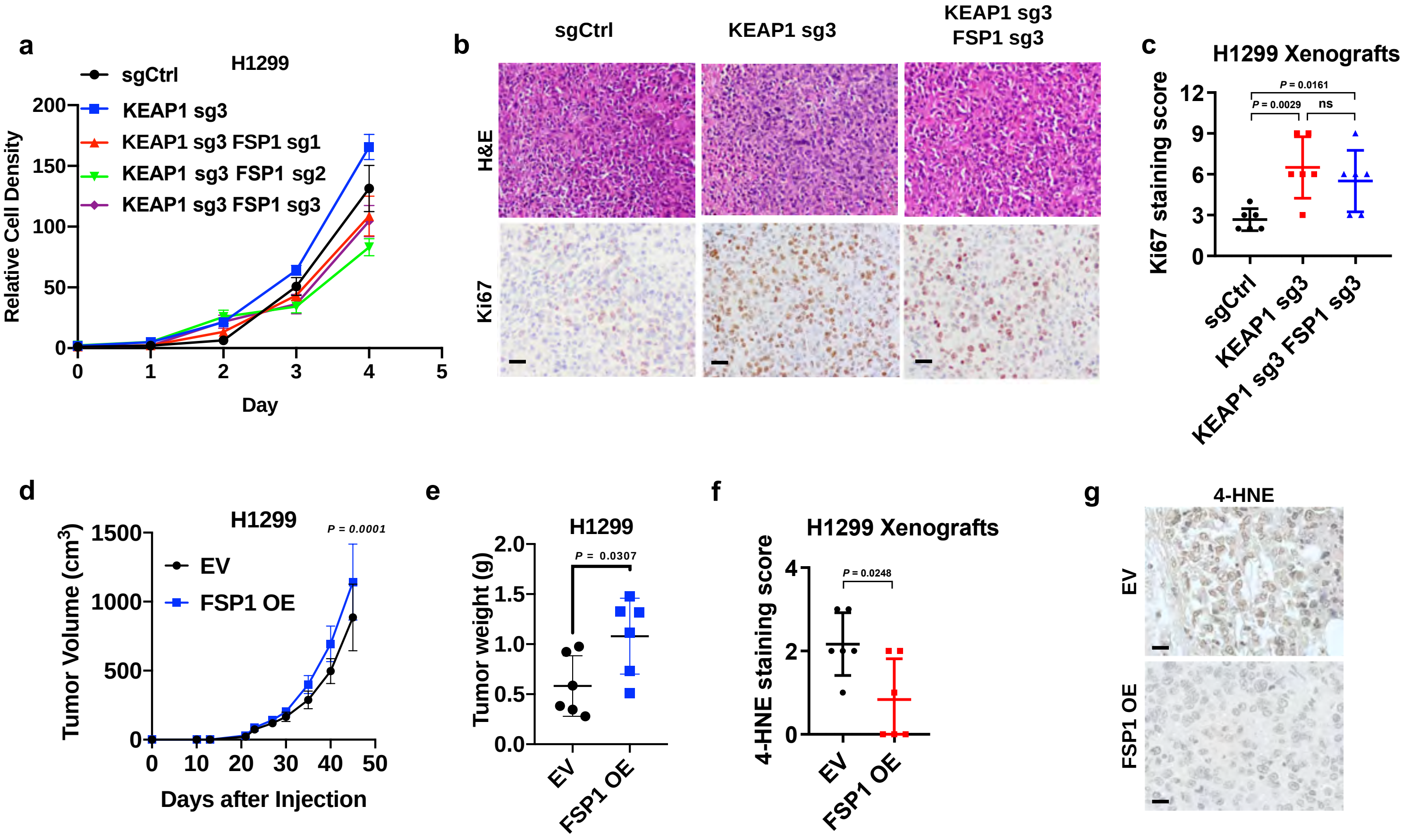
Supplementary Figure 4



Supplementary Figure 4. KEAP1 regulates FSP1 through NRF2-mediated transcription.

a, **b** NRF2 ChIP-seq profiles in lymphoblastoid, A549, IMR90, HepG2 and Hela S3 cell lines (GEO datasets: GSE37589, GSE91894, GES91565, GSE91809 and GSE91997). **c** Unsupervised clustering analyses of FSP1 and NRF2 target genes in multiple cancers. **d** Correlation analyses of FSP1 and classical NRF2 target genes in TCGA-LUAD. **e** Protein levels of FSP1 and NRF2 in H1299 cells treated with TBHQ. **f** qRT-PCR analysis of *FSP1* in H1299 treated with TBHQ. **g** qRT-PCR analysis of *FSP1* in *KEAP1 NRF2* DKO H1299 cells. **h** FACS panels of PI staining from three independent repeats showing cell death upon RSL3 treatment in *KEAP1 NRF2* DKO H1299 cells overexpressing FSP1. For **f** and **j**, P value was determined by two-tailed unpaired Student's t test. Error bars are (if mentioned otherwise) mean +/- SD; *n* =3; ns, not significant. Source data are provided as a Source Data file.

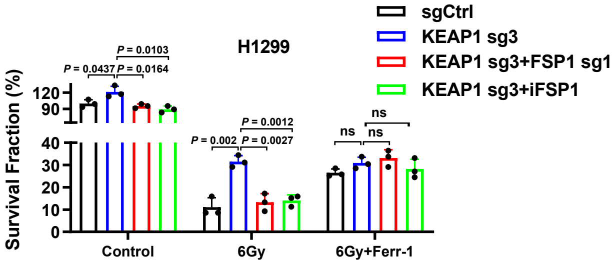
Supplementary Figure 5



Supplementary Figure 5. FSP1 promotes tumorigenesis in lung cancer upon *KEAP1* deletion

a Proliferation rates of *KEAP1 FSP1* DKO H1299 cell lines. Error bars are means +/- SD, *n* = 3. **b** Representative images of hematoxylin and eosin and Ki67 immunohistochemical staining in *KEAP1 FSP1* DKO H1299 xenograft tumors. Scale bars, 50 µm. **c** Immunohistochemistry scoring of Ki67 staining in *KEAP1 FSP1* DKO H1299 xenograft tumor sections. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. **d** Tumor volumes of empty vector (EV) and FSP1-overexpressing (OE) H1299 xenograft tumors. Error bars are mean +/− SD; *n* = 6 tumors. **e** End point weights of EV and FSP1 OE H1299 xenograft tumors. Error bars are mean +/− SD; *n* = 6 tumors. **f, g** Immunochemistry staining scoring (**f**) and representative images (**g**; scale bars, 20 µm) of 4-HNE in indicated H1299 xenograft tumor sections. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. Data are presented as (if mentioned otherwise) mean +/- SD; *n* =3. P value was determined by two-tailed unpaired Student's t test; ns, not significant. Source data are provided as a Source Data file.

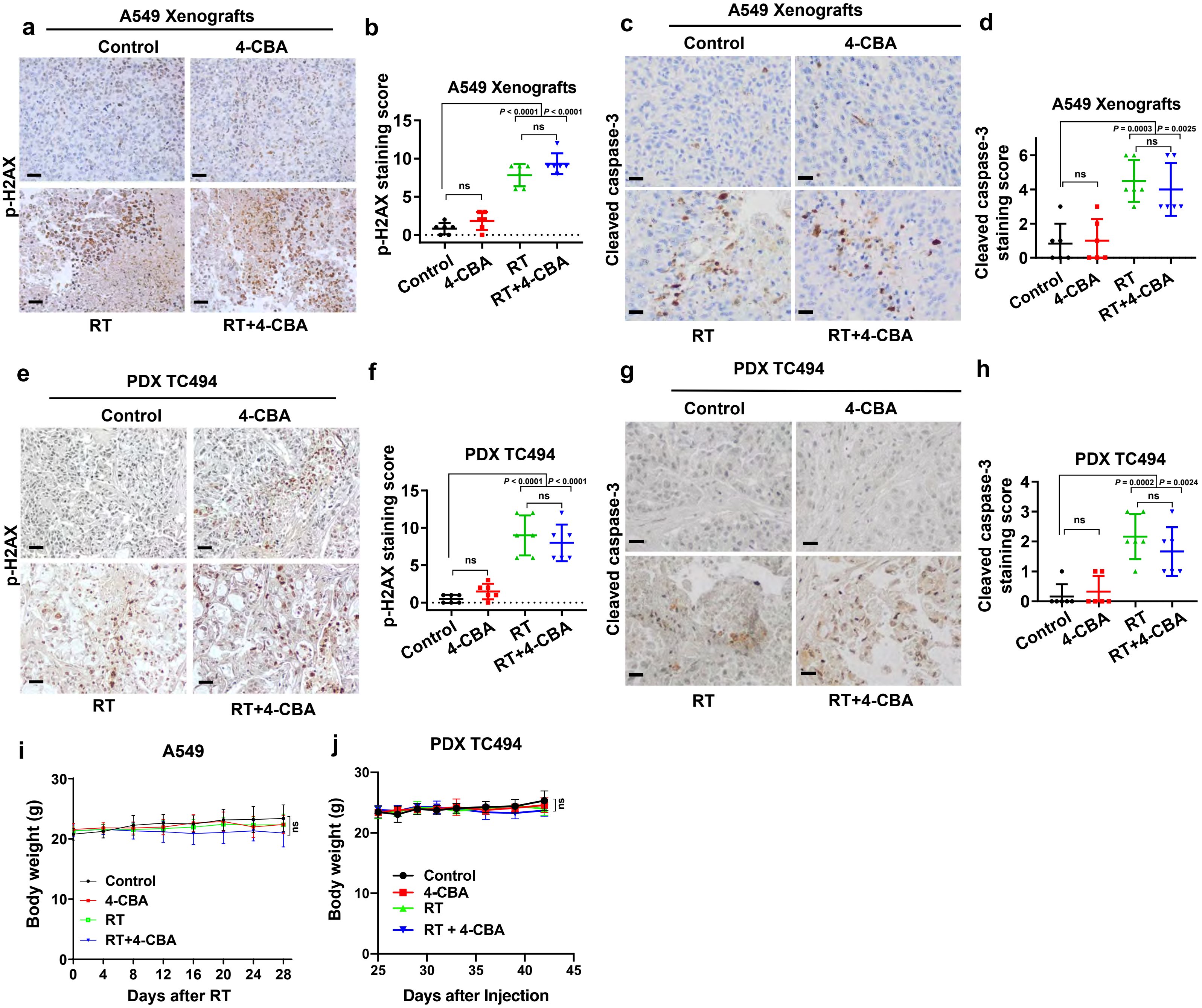
Supplementary Figure 6



Supplementary Figure 6. FSP1 inhibition sensitizes *KEAP1* deficient lung cancer cells to radiation through ferroptosis.

Survival fraction of *KEAP1* KO H1299 cells with genetic or pharmacological inhibition of FSP1 treated with 6 Gy X-ray irradiation alone or with ferrostatin-1 pretreatment for 24h. Data are presented as (if mentioned otherwise) mean +/- SD; *n* =3. P value was determined by two-tailed unpaired Student’s t test; ns, not significant. Source data are provided as a Source Data file.

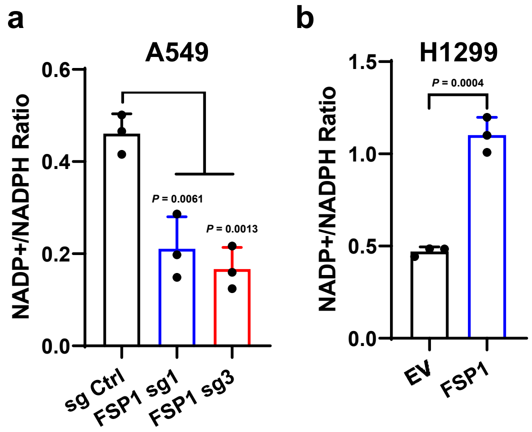
Supplementary Figure 7



Supplementary Figure 7. Inhibiting CoQ synthesis reverses radioresistance in *KEAP1* deficient or mutant lung cancers.

a, b Representative images (**a**; scale bars, 20 μm) and scores (**b**) of IHC staining for p-H2AX in A549 xenograft tumors with indicated treatments. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. **c, d** Representative images (**c**; scale bars, 20 μm) and scores (**d**) of IHC staining for cleaved caspase-3 in A549 xenograft tumors with indicated treatments. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. **e, f** Representative images (**e**; scale bars, 20 μm) and scores (**f**) of IHC staining for p-H2AX in PDX TC494 tumors with indicated treatments. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. **g, h** Representative images (**g**; scale bars, 20 μm) and scores (**h**) of IHC staining for cleaved caspase-3 in PDX TC494 tumors with indicated treatments. Error bars are means +/- SD, *n* = 6 randomly selected magnification fields. **i, j** Mice weights of xenografts derived from A549 (**i**) or PDX TC494 tumors (**j**) grown at different time points treated with either 10 Gy X-ray irradiation or 4-CBA alone, or combined treatement of radiation and 4-CBA. Error bars are means +/- SD, *n* = 5-8 mice. P value was determined by two-tailed unpaired Student's t test; ns, not significant. Source data are provided as a Source Data file.

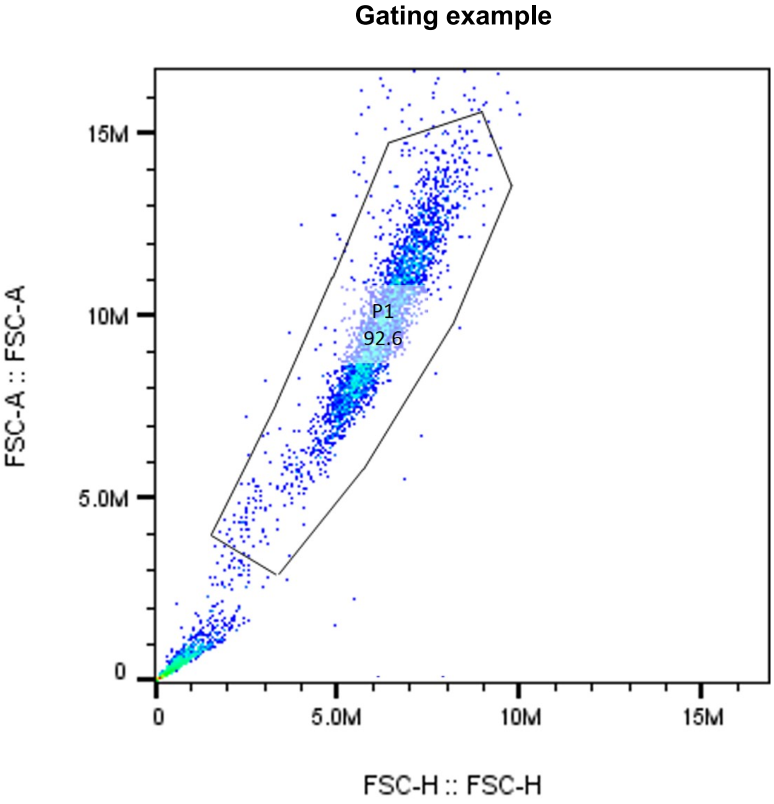
Supplementary Figure 8



Supplementary Figure 8. Inhibiting CoQ synthesis reverses radioresistance in *KEAP1* deficient or mutant lung cancer.

a, b $NADP^+$/NADPH ratio in A549 *FSP1* KO cells (**a**) and H1299 *FSP1* overexpressing cells (**b**). Error bars are presented as mean +/- SD; *n* =3 independent samples. P value was determined by two-tailed unpaired Student's t test. Source data are provided as a Source Data file.

Supplementary Figure 9



Supplementary Figure 9. Gating strategies for lipid peroxidation analysis through flow cytometry.

Supplementary Table 1. Ubiquinone metabolic pathway enrichment

Ubiquinone metabolic pathway enrichment		
	log2FC	p value
COQ6	0.313	0.00000162
COQ3	0.4504	1.77E-09
COQ5	0.18560449	0.00320091
PDSS1	0.38256335	0.0000134
COQ9	0.34665797	4.23E-08
FSP1	1.12606245	1.70E-38
COQ2	0.17774408	0.00394329
NDUFA9	0.40682899	1.13E-08
COQ4	0.17424572	0.00921812
COQ10B	0.20002756	0.0000697
FDXR	0.18537058	0.04554662
COQ7	0.14756895	0.00784763

Supplementary Table 2. Oligos sequences and quantitative reverse transcription PCR primer sequences

Oligos sequences	
Control-sgRNA-F	CACCGGCACTACCAGAGCTAACTCA
Control-sgRNA-R	AAACTGAGTTAGCTCTGGTAGTGCC
KEAP1-sgRNA#1-F	CACCGCTTGTGGGCCATGAACTGGG
KEAP1-sgRNA#1-R	AAACCCCAGTTCATGGCCCACAAGC
KEAP1-sgRNA#2-F	CACCGTGTGTCCTCCACGTCATGAA
KEAP1-sgRNA#2-R	AAAC TTCATGACGTGGAGGACACAC
KEAP1-sgRNA#3-F	CACCGGAGGACACACTTCTCGCCCA
KEAP1-sgRNA#3-R	AAAC TGGGCGAGAAGTGTGTCCTCC
NRF2-sgRNA#3-F	CACCGTTACAACTAGATGAAGAGAC
NRF2-sgRNA#3-R	AAACGTCTCTTCATCTAGTTGTAAC
NRF2-sgRNA#4-F	CACCGCAGATCCACTGGTTTCTGAC
NRF2-sgRNA#4-R	AAACGTCAGAAACCAGTGGATCTGC
SLC7A11-sgRNA#2-F	CACCGATGAGCTTGATCGCAAGTTC
SLC7A11-sgRNA#2-R	AAACGAACTTGCGATCAAGCTCATC
SLC7A11-sgRNA#3-F	CACCGAAGTATTACGCGGTTGCCAC
SLC7A11-sgRNA#3-R	AAACGTGGCAACCGCGTAATACTTC
GPX4-sgRNA-F	CACCGGGTGAAGCGCTACGGACCCA
GPX4-sgRNA-R	AAACTGGGTCCGTAGCGCTTCACCC
FSP1-sgRNA-F#1-F	CACCGCCACGATGAACCGTGAGCGC
FSP1-sgRNA-R#1-R	AAACGCGCTCACGGTTCATCGTGGC
FSP1-sgRNA-F#3-F	CACCGCATCTTGGGCGTCCTCACGT
FSP1-sgRNA-R#3-R	AAACACGTGAGGACGCCCAAGATGC
COQ2-sgRNA-F	CACCGATGCTGGGCTCGCGAGCCGC
COQ2-sgRNA-R	AAACGCGGCTCGCGAGCCCAGCATC
KEAP1-sgRNA#3-mutation1F	CCTACACGGCCTCCATCTCGATGGGCGA
KEAP1-sgRNA#3-mutation1R	TCGCCCATCGAGATGGAGGCCGTGTAGG
KEAP1-sgRNA#3-mutation2F	ATGGGCGAAAAGTGTGTCCTCCACGTC
KEAP1-sgRNA#3-mutation2R	GACGTGGAGGACACACTTTTCGCCCAT
KEAP1 G333C-mutant 1F	TACACCGCGGGCTGCTACTTCCGACAGT
KEAP1 G333C-mutant 1R	ACTGTCGGAAGTAGCAGCCCGCGGTGTA
Quantitative Reverse Transcription PCR primer sequences	
SLC7A11-F	ATGCAGTGGCAGTGACCTTT
SLC7A11-R	GGCAACAAAGATCGGAACTG
GPX4-F	ACAAGAACGGCTGCGTGGTGAA
GPX4-R	GCCACACACTTGTGGAGCTAGA
FSP1-F	GAATCCCAGTGTGTGAGCGA
FSP1-R	GATGCACACCCCTGTGTCAA
NRF2-F	CAGCGACGGAAAGAGTATGA
NRF2-R	TGGGCAACCTGGGAGTAG
PTGS2-F	CTGATGATTGCCCGACTCCC
PTGS2-R	TCGTAGTCGAGGTCATAGTTC
Actin-F	CGGAACCGCTCATTGCC
Actin-R	ACCCACACTGTGCCCATCTA