Supplementary Information

SLC7A11 expression level dictates differential responses to oxidative stress in cancer cells

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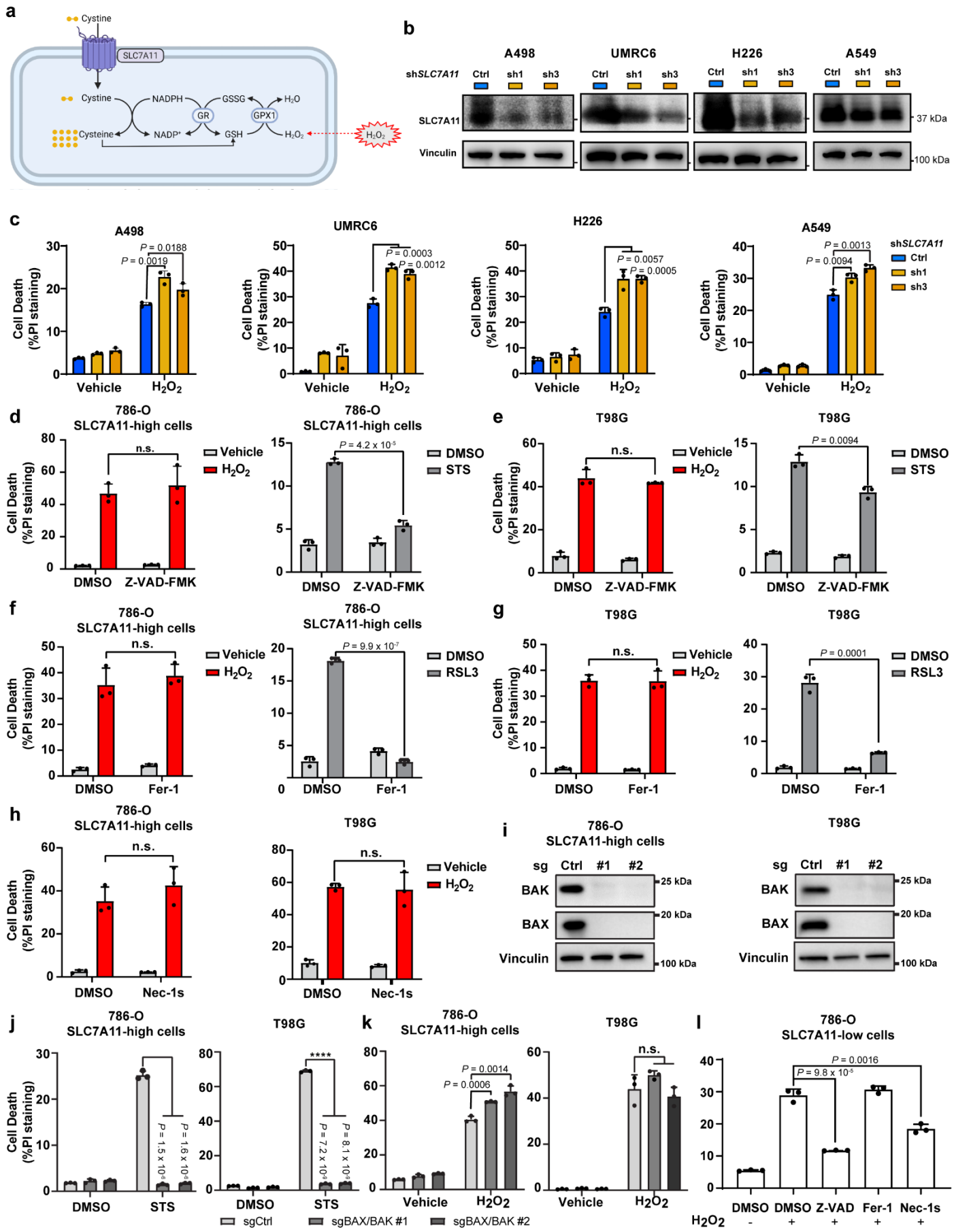
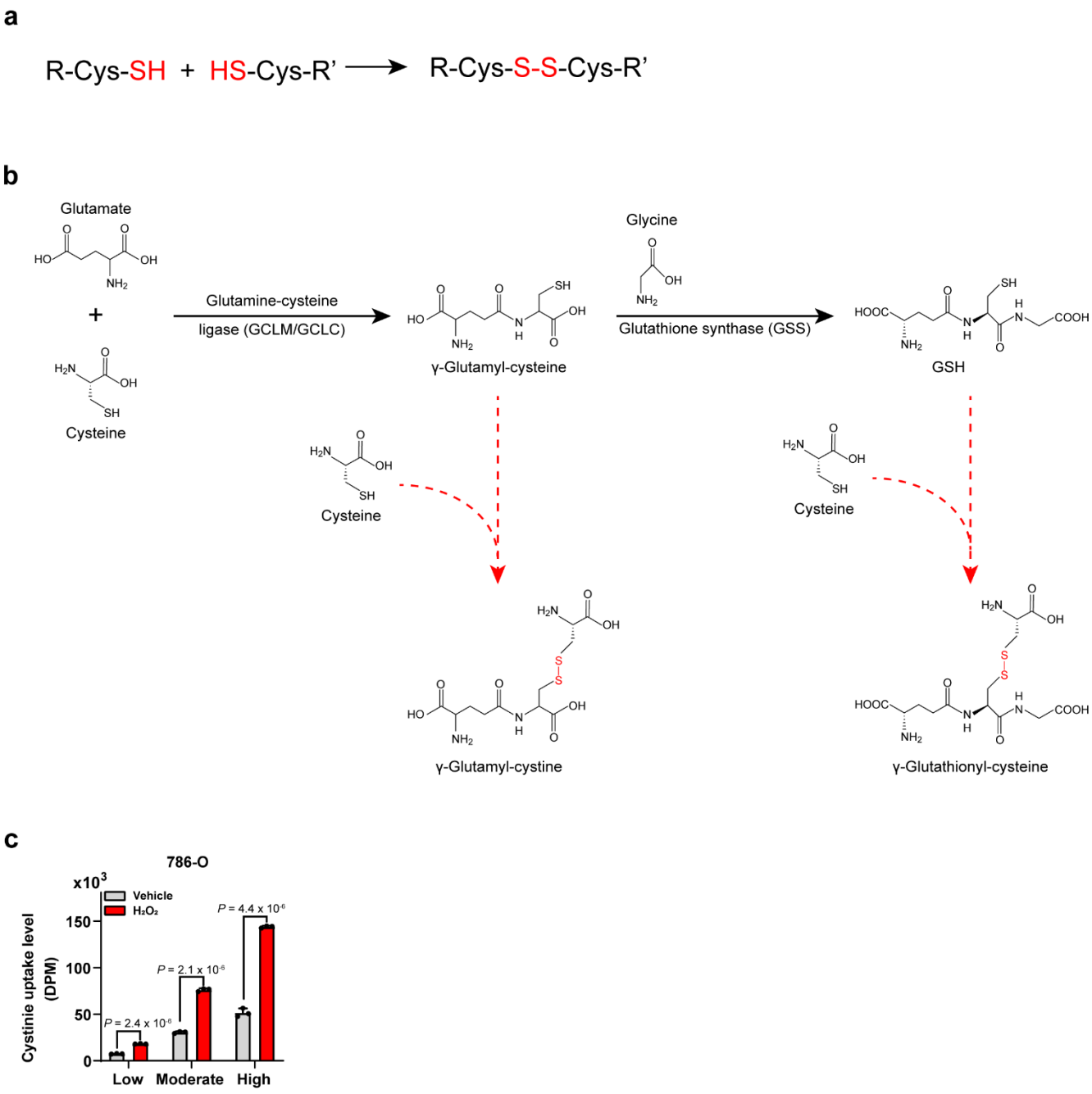
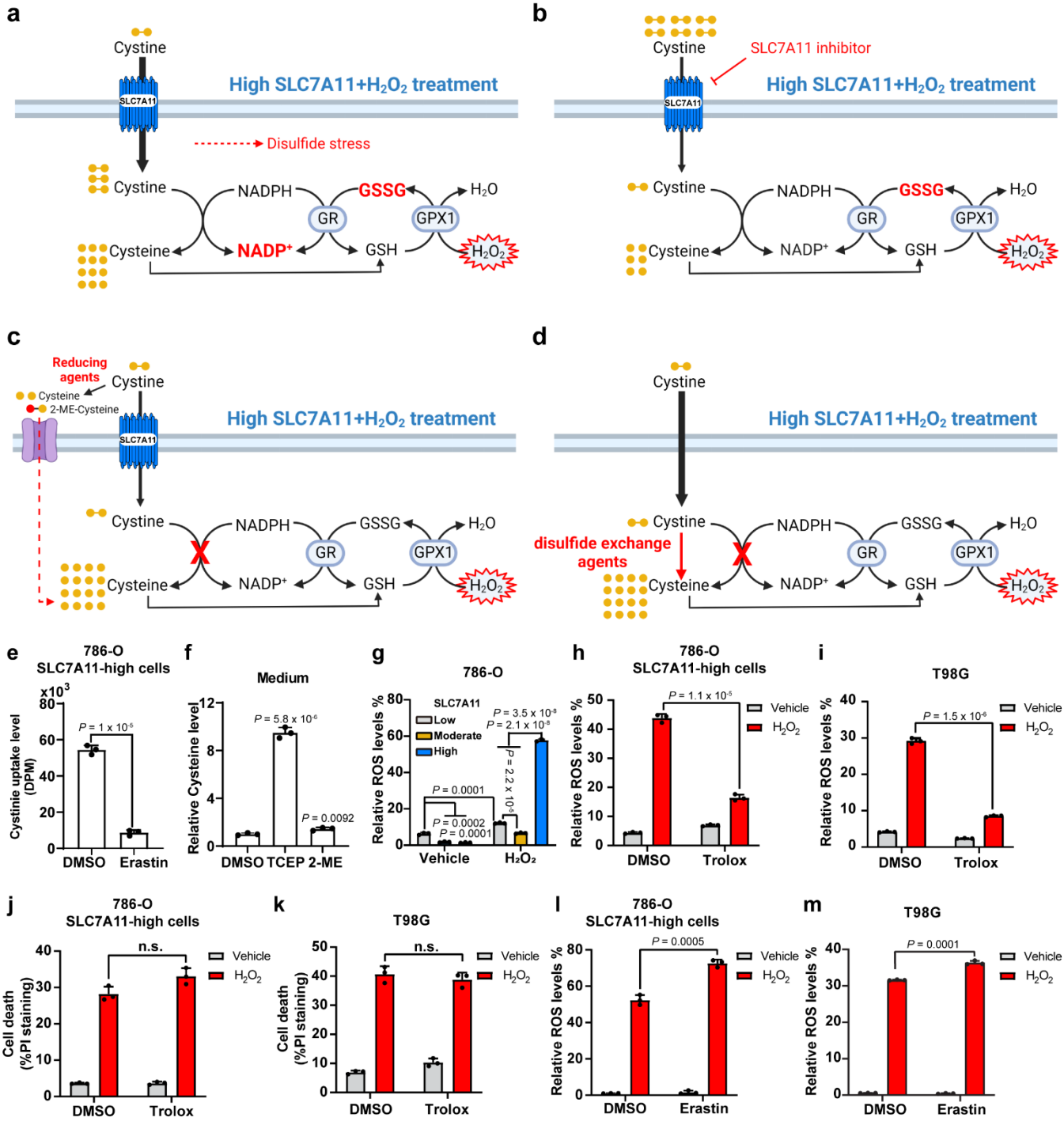

a
Cystine
SLC7A11
Cystine
NADPH
GSSG
H2O
GR
GPX1
Cysteine
NADP+
GSH
H2O2
H2O2
b
A498
UMRC6
H226
A549
shSLC7A11
Ctrl
sh1
sh3
SLC7A11
37 kDa
Vinculin
100 kDa
c
A498
UMRC6
H226
A549
Cell Death (%PI staining)
P = 0.0188
P = 0.0019
P = 0.0003
P = 0.0012
P = 0.0057
P = 0.0005
P = 0.0013
P = 0.0094
shSLC7A11
Ctrl
sh1
sh3
Vehicle
H2O2
d
786-O
SLC7A11-high cells
n.s.
Vehicle
H2O2
P = 4.2 x 10-5
DMSO
STS
DMSO
Z-VAD-FMK
e
T98G
n.s.
P = 0.0094
f
786-O
SLC7A11-high cells
n.s.
P = 9.9 x 10-7
DMSO
RSL3
DMSO
Fer-1
g
T98G
n.s.
P = 0.0001
h
786-O
SLC7A11-high cells
T98G
n.s.
DMSO
Nec-1s
i
786-O
SLC7A11-high cells
T98G
sg
Ctrl
#1
#2
BAK
BAX
Vinculin
25 kDa
20 kDa
100 kDa
j
786-O
SLC7A11-high cells
T98G
P = 1.5 x 10-6
P = 1.6 x 10-6
P = 7.2 x 10-6
P = 8.1 x 10-6
****
DMSO
STS
sgCtrl
sgBAX/BAK #1
sgBAX/BAK #2
k
786-O
SLC7A11-high cells
T98G
P = 0.0014
P = 0.0006
n.s.
Vehicle
H2O2
l
786-O
SLC7A11-low cells
P = 0.0016
P = 9.8 x 10-5
DMSO
DMSO
Z-VAD
Fer-1
Nec-1s
H2O2
-
+
+
+
+

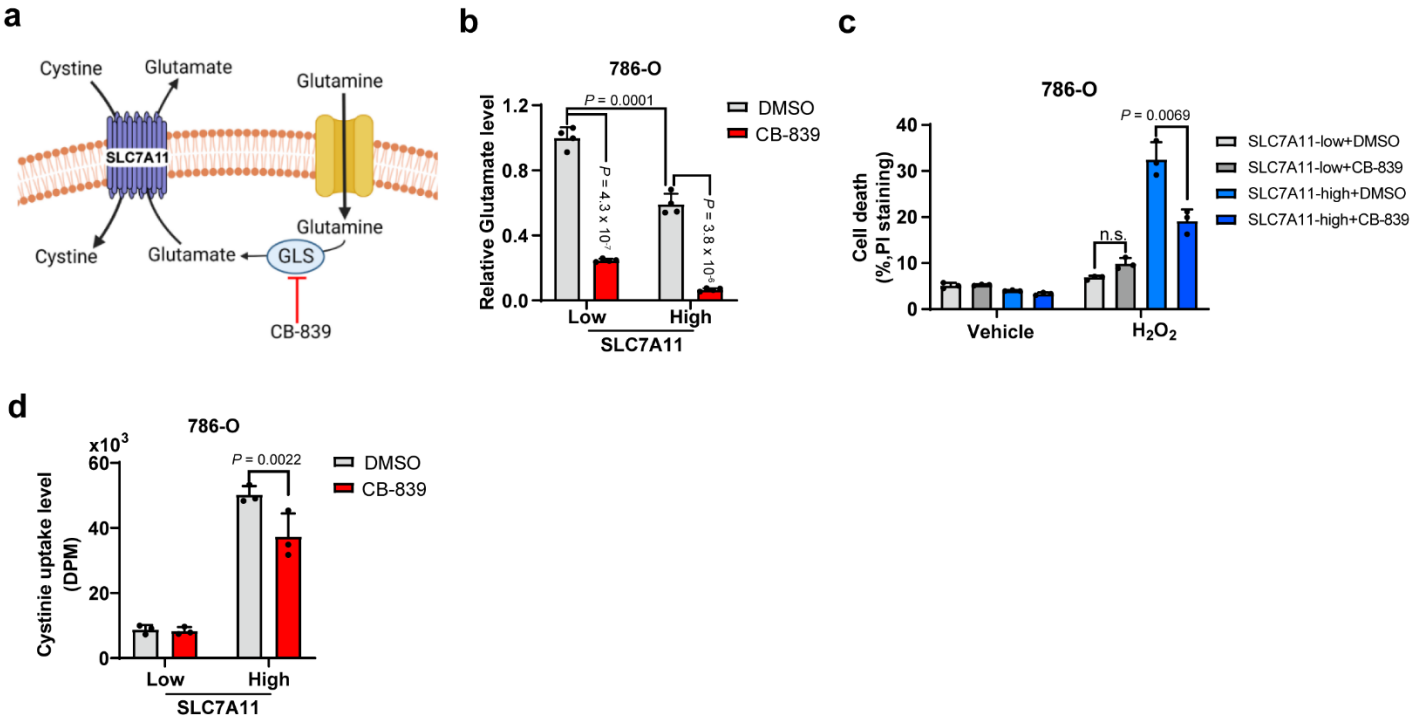
Supplementary Figure 1. Cell death induced by high SLC7A11 expression under H_2O_2 treatment is not apoptosis, necroptosis, or ferroptosis. a, Simplified schematic showing how cells detoxify H_2O_2 through the SLC7A11-glutathione (GSH)-glutathione peroxidase 1 (GPX1) axis. **b**, Protein levels of SLC7A11 in indicated cell lines with shCtrl and *SLC7A11* knockdown. **c,** Cell death in response to 1 mM H_2O_2 treatment in A498, UMRC6, H226 and A549 cells with indicated genotypes for 24 hours was measured using PI staining. **d-e**, Cell death in response to co-treatment with 1 mM H_2O_2 for 6 hours (left), or 30 μM staurosporine (STS) for 24 hours (right), and 10 μM Z-VAD-FMK in SLC7A11-high 786-O cells **(d)** and T98G cells **(e)** was measured using PI staining. **f**. Cell death in response to co-treatment with 1 mM H_2O_2 for 6 hours (left) or 0.2 μM RSL3 for 16 hours (right) with 5 μM ferrostatin-1 (Fer-1) in SLC7A11-high 786-O cells was measured using PI staining. **g**, Cell death in response to co-treatment with 1 mM H_2O_2 (left) or 1 μM RSL3 (right) for 24 hours with 5 μM ferrostatin-1 in T98G cells was measured using PI staining. **h**, Cell death under co-treatment with 1 mM H_2O_2 and 2 μM necrostatin-1s (Nec-1s) in SLC7A11-high 786-O (left) or T98G (right) cells was measured using PI staining. **i**, Protein levels of BAK and BAX in sgCtrl and *BAX/BAK* double knockout SLC7A11-high 786-O (left) or T98G (right) cells. **j-k**, Cell death in response to treatment with 30 μM STS **(j)** or 1 mM H_2O_2 **(k)** in SLC7A11-high 786-O (left) or T98G (right) cells with indicated genotypes was measured using PI staining. **l,** Cell death under co-treatment with 1 mM H_2O_2 and Z-VAD-FMK, Fer-1 or Nec-1s for 20 hrs. Data were presented as mean ± SD; n = 3. n indicates independent repeats. P value was determined by two-tailed unpaired Student's t test. n.s., not significant. Source data are provided as a Source Data file. GR, glutathione reductase; GSSG, glutathione disulfide; NADPH, nicotinamide adenine dinucleotide phosphate.



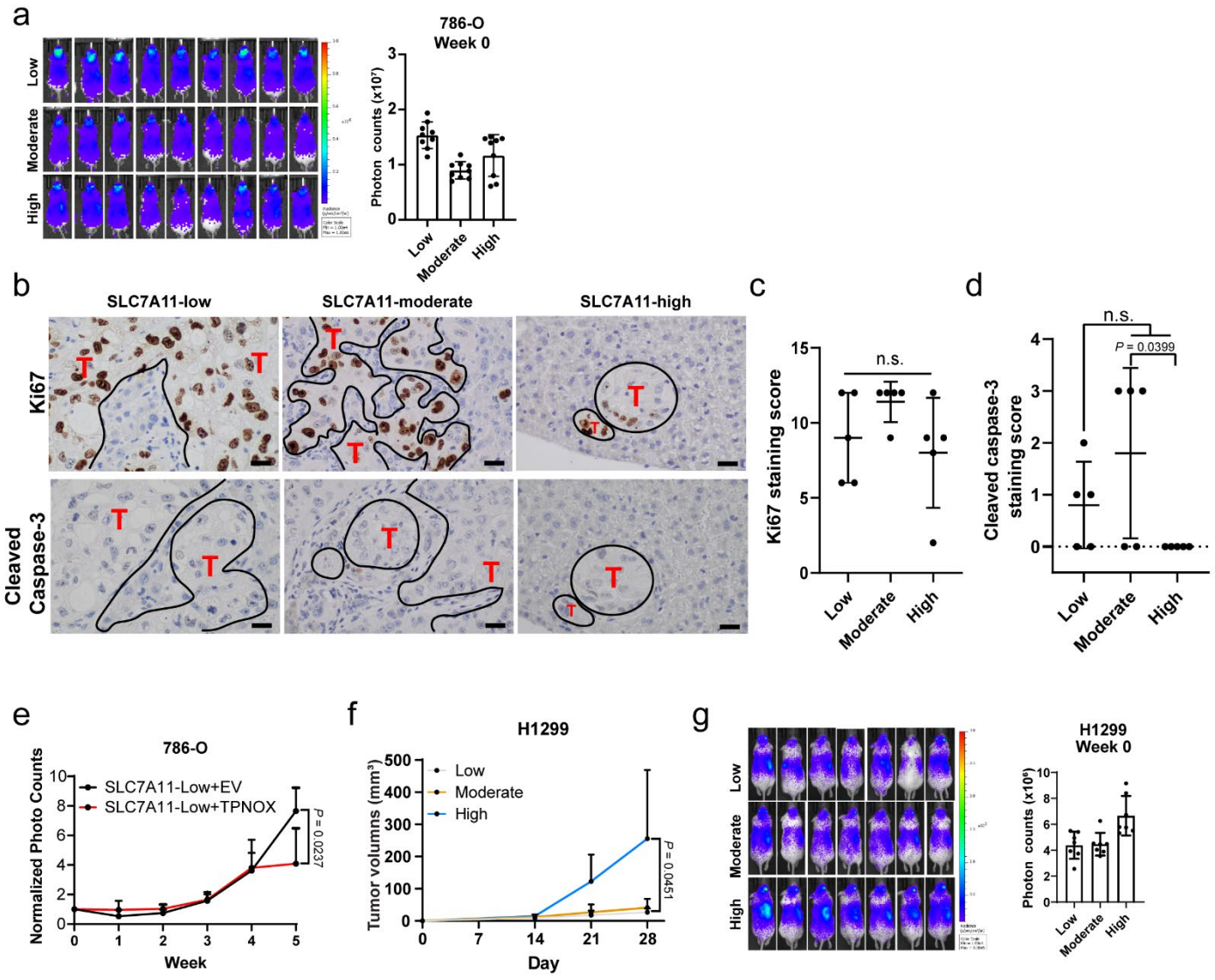
Supplementary Figure 2. Schematic of disulfide molecule formation. a, Diagram illustrating disulfide bond formation. **b**, Diagram illustrating the synthesis of various cystine-derived disulfide molecules. **c,** Cystine uptake levels in SLC7A11-low, -moderate, and -high 786-O cells treated with vehicle or 1 mM H_2O_2 for 1hr. Data were presented as mean ± SD; n = 3. n indicates independent repeats. P value was determined by two-tailed unpaired Student's t test. Source data are provided as a Source Data file.


a
Cystine
SLC7A11
High SLC7A11+H2O2 treatment
Disulfide stress
Cystine
NADPH
GSSG
H2O
GR
GPX1
Cysteine
NADP+
GSH
H2O2
b
Cystine
SLC7A11 inhibitor
SLC7A11
High SLC7A11+H2O2 treatment
Cystine
NADPH
GSSG
H2O
GR
GPX1
Cysteine
NADP+
GSH
H2O2
c
Reducing agents
Cystine
Cysteine
2-ME-Cysteine
SLC7A11
High SLC7A11+H2O2 treatment
Cystine
NADPH
GSSG
H2O
GR
GPX1
Cysteine
NADP+
GSH
H2O2
d
Cystine
High SLC7A11+H2O2 treatment
Cystine
disulfide exchange agents
NADPH
GSSG
H2O
GR
GPX1
Cysteine
NADP+
GSH
H2O2
e
786-O
SLC7A11-high cells
x10^3
Cystinie uptake level (DPM)
P = 1 x 10-5
DMSO
Erastin
f
Medium
Relative Cysteine level
P = 5.8 x 10-6
P = 0.0092
DMSO
TCEP
2-ME
g
786-O
SLC7A11
Low
Moderate
High
Relative ROS levels %
P = 3.5 x 10-8
P = 2.1 x 10-8
P = 2.2 x 10-8
P = 0.0001
P = 0.0002
P = 0.0001
Vehicle
H2O2
h
786-O
SLC7A11-high cells
Relative ROS levels %
P = 1.1 x 10-5
Vehicle
H2O2
DMSO
Trolox
i
T98G
Relative ROS levels %
P = 1.5 x 10-6
Vehicle
H2O2
DMSO
Trolox
j
786-O
SLC7A11-high cells
Cell death (%PI staining)
n.s.
Vehicle
H2O2
DMSO
Trolox
k
T98G
Cell death (%PI staining)
n.s.
Vehicle
H2O2
DMSO
Trolox
l
786-O
SLC7A11-high cells
Relative ROS levels %
P = 0.0005
Vehicle
H2O2
DMSO
Erastin
m
T98G
Relative ROS levels %
P = 0.0001
Vehicle
H2O2
DMSO
Erastin

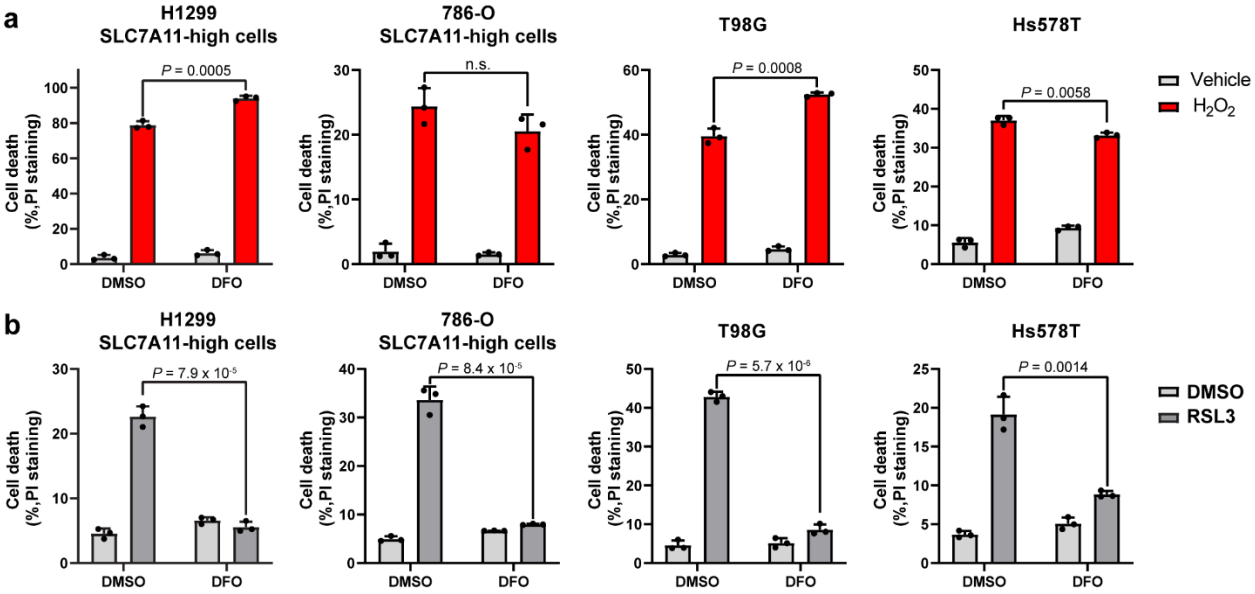
Supplementary Figure 3. Uncoupling between reactive oxygen species (ROS) and cell death induced by high SLC7A11 expression under H_2O_2 treatment. a-d, Simplified schematics illustrating redox system alterations in SLC7A11-high cells under H_2O_2 treatment with no additional treatment (**a**), or treatment with SLC7A11 inhibitors (**b**), reducing agents (**c**), or disulfide-exchange agents (**d**). **e**, Cystine uptake level in SLC7A11-high 786-O cells treated with or without 5 μM erastin. **f**, Cysteine level in medium treated with or without 1 mM TCEP or 2-ME. **g**, Relative ROS levels in SLC7A11-low, -moderate, and -high 786-O cells treated with vehicle or 1 mM H_2O_2. **h, i,** Relative ROS levels in SLC7A11-high 786-O (**h**) or T98G (**i**) cells treated with vehicle or 1 mM H_2O_2 with or without 0.5 mM Trolox. **j, k,** Cell death in response to treatment with 1 mM H_2O_2 with or without 0.5 mm Trolox in SLC7A11-high 786-O (**j**) or T98G (**k**) cells measured using PI staining. **l**, **m**, Relative ROS levels in SLC7A11-high 786-O cells (**l**) or T98G (**m**) cells treated with vehicle or 1 mM H_2O_2 (1 mM) with or without 5 μM erastin. Data were presented as mean ± SD; n = 3. n indicates independent repeats. P value was determined by two-tailed unpaired Student's t test. n.s., not significant. Source data are provided as a Source Data file. GPX1, glutathione peroxidase 1; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; NADPH, nicotinamide adenine dinucleotide phosphate.



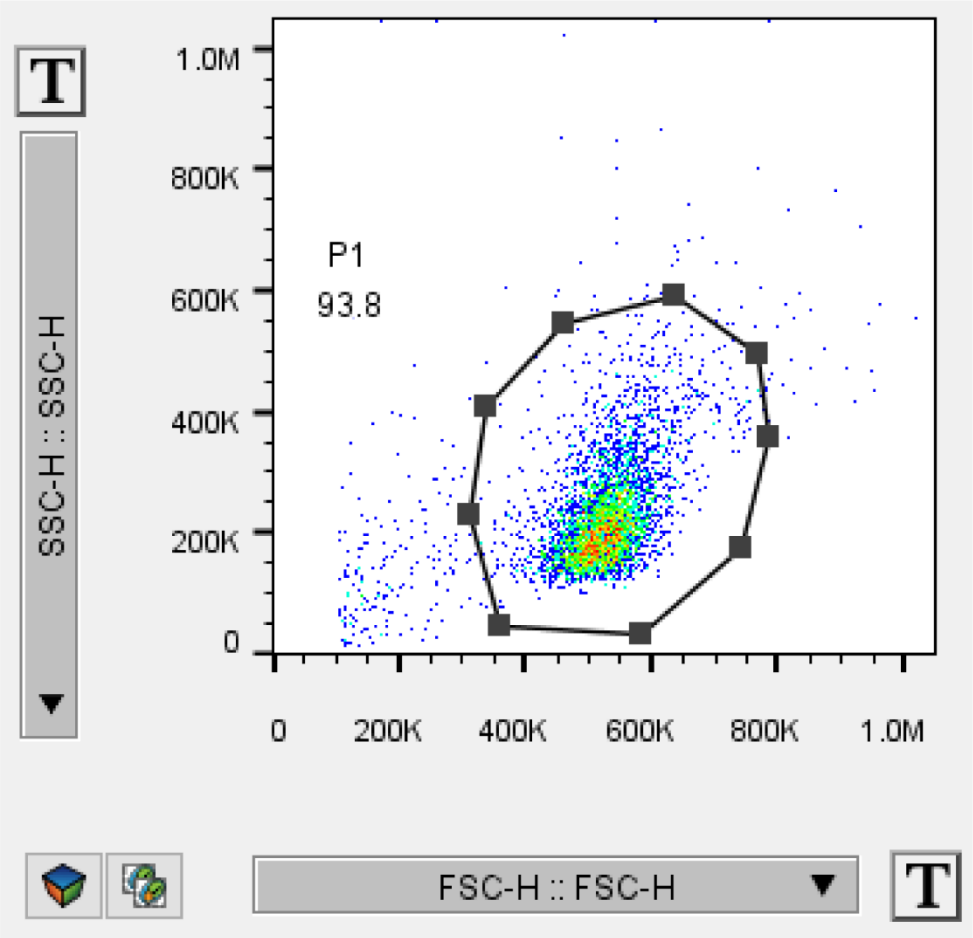
Supplementary Figure 4. Glutaminase inhibition decreases intracellular glutamate levels and suppresses H_2O_2–induced cell death in SLC7A11-high cells. a, Simplified schematic illustrating how glutamine metabolism is involved in SLC7A11-mediated cystine uptake. **b,** Measurement of intracellular glutamate level in SLC7A11-low and -high 786-O cells treated with 5 μM CB-839 for 1 hr (n = 4). **c**, Cell death in response to co-treatment with 1 mM H_2O_2 and 5 μM CB-839 for 6 hr in in SLC7A11-low and -high 786-O cells (n = 3)**. d,** Cystine uptake levels in SLC7A11-low and -high 786-O cells treated with 5 μM CB-839 for 1 hr (n = 3). Data were presented as mean ± SD. n indicates independent repeats. P value was determined by two-tailed unpaired Student's t test. Source data are provided as a Source Data file.


a
Low
Moderate
High
786-O
Week 0
Photon counts (x10⁷)
b
SLC7A11-low
SLC7A11-moderate
SLC7A11-high
Ki67
Cleaved Caspase-3
T
c
Ki67 staining score
n.s.
d
Cleaved caspase-3 staining score
n.s.
P = 0.0399
e
786-O
Normalized Photo Counts
SLC7A11-Low+EV
SLC7A11-Low+TPNOX
P = 0.0237
Week
f
H1299
Tumor volumns (mm³)
Low
Moderate
High
P = 0.0451
Day
g
H1299
Week 0
Photon counts (x10⁶)

Supplementary Figure 5. *In vivo* imaging of cancer cells after intracardiac injection. a, Images of bioluminescence in mice 30 min after intracardiac injection with SLC7A11-low, -moderate, and -high 786-O cells (left) and statistical analysis of whole-body photon flux (right) (n = 9 mice). **b**, Representative images of immunohistochemical staining (Ki67 and cleaved caspase-3) of livers with tumor metastasis derived from SLC7A11-low, -moderate, or -high 786-O cells. "T" stands for tumor cells. Scale bars, 20 μm. **c, d,** Immunohistochemical staining scores for Ki67 (**c**) and cleaved caspase-3 (**d**) in livers with tumor metastasis derived from SLC7A11-low, -moderate, or -high 786-O cells. **e,** Quantification of photon flux (photons per second) in mice normalized to day 0 after intracardiac injection of SLC7A11-high 786-O cells with EV or TPNOX overexpression (n = 5 mice). **f,** Measurement of tumor volumes in SLC7A11-low, -moderate, and -high H1299 xenograft tumors at different time points (days) after subcutaneous injection (n = 5 mice for low/moderate and n = 4 mice for high). **g,** Images of bioluminescence in mice 30 min after intracardiac injection with SLC7A11-low, -moderate, and -high H1299 cells (left) and statistical analysis of whole-body photon flux (right) (n = 7 mice). Data were presented as mean ± SD. n indicates mice or independent repeats. P value was determined by two-tailed unpaired Student's t test. n.s., not significant. Source data are provided as a Source Data file.



Supplementary Figure 6. Iron chelator treatment does not suppress cell death induced by high SLC7A11 expression under H_2O_2 treatment. a, Cell death in response to treatment with 1 mM H_2O_2 with or without 200 nM deferoxamine (DFO) in SLC7A11-high H1299, SLC7A11-high 786-O, T98G, and Hs578T cells measured using propidium iodide (PI) staining. **b**, Cell death in response to treatment with RSL3 with or without 200 nM DFO in SLC7A11-high H1299, SLC7A11-high 786-O, T98G, and Hs578T cells measured using PI staining. Data were presented as mean ± SD. n indicates independent repeats. P value was determined by two-tailed unpaired Student's t test. n.s., not significant. Source data are provided as a Source Data file.



Supplementary Figure 7. Gating strategies for flow cytometry. Initial cell population gating (FSC-H vs. SSC-H) was used to make sure only single cells were used for analysis.

Supplementary Table 1. Oligo sequences for CRISPR-Cas9 knockout.

sgRNA name	sgRNA sequence
sgCtrl	GGCACTACCAGAGCTAACTCA
sg*SLC7A11* #3	GAAGTATTACGCGGTTGCCAC
sg*SLC7A11* #4	GGTGTTCTGGAGCACGCCCTT
sg*BAX* #1	GTGAGCAGATCATGAAGACAG
sg*BAX* #2	GCGAGTGTCTCAAGCGCATCG
sg*BAK* #1	GGCAGGTAGCCCAGGACACAG
sg*BAK* #2	GGGAACTCTGAGTCATAGCGT
sg*GPX1*	GGGGGTCGGTCATAAGCGCG