Figure S1

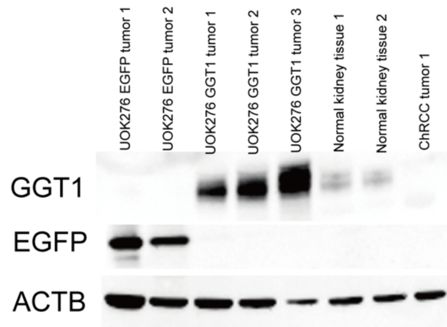

Figure S1. GGT1 protein expression in UOK276 xenograft tumors.

(**A**) Immunoblot of GGT1 protein expression in UOK276 EGFP and GGT1 xenograft tumors, human normal kidneys and one ChRCC tumor.

Figure S2

A

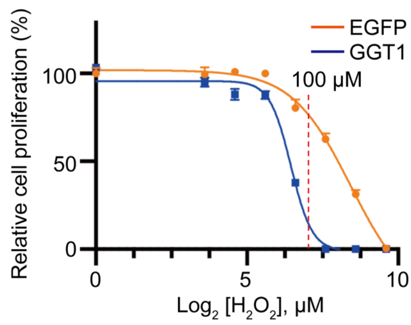

Figure S2. Sensitivity of ChRCC cells to H_2O_2. (**A**) Proliferation (crystal violet) of UOK276 cells following 48 hours treatment with increasing doses of H_2O_2. Error bars represent mean ± SD. Statistical significance was calculated using one way ANOVA with Tukey's multiple comparisons test.

Figure S3

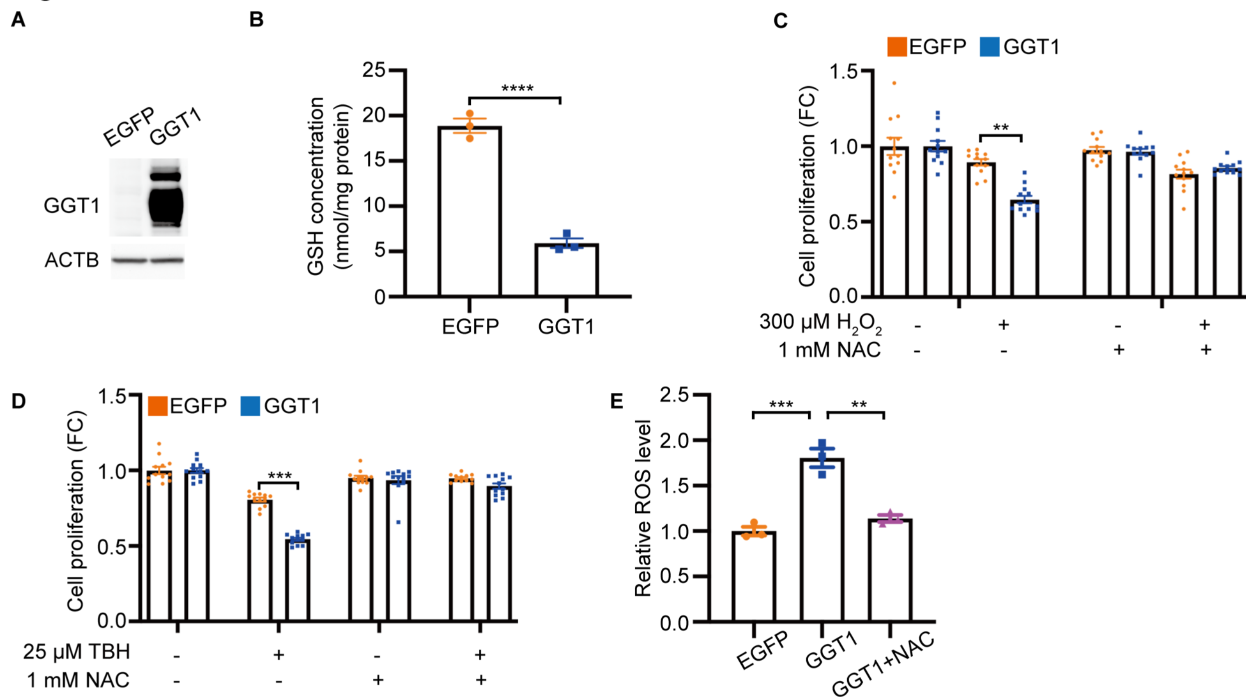

Figure S3. GGT1 overexpression reduces GSH levels and promotes the sensitivity of HEK293 cells to hydrogen peroxide (H_2O_2). (**A**) GGT1 protein levels in HEK293T cells expressing EGFP (control) or GGT1. (**B**) GSH concentration in HEK293T cells expressing EGFP and GGT1 (n=3 biological replicates per condition). (**C**) Proliferation (crystal violet) of HEK293T cells expressing EGFP or GGT1 treated with H_2O_2 (300 μM) or NAC (1 mM) alone or in combination for 48 hrs. (**D**) Proliferation (crystal violet) of HEK293T cells expressing EGFP or GGT1 treated with TBH (25 μM) or NAC (1 mM) alone or in combination for 48 hrs. (**E**) ROS level (Deep Red assay) in HEK293T cells with EGFP or GGT1 expression, and with NAC (1 mM, 24 hours) (n=3 biological replicates per condition). Error bars represent mean ± SD. Statistical significance was calculated using one way ANOVA by Tukey's multiple comparisons test. **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Figure S4

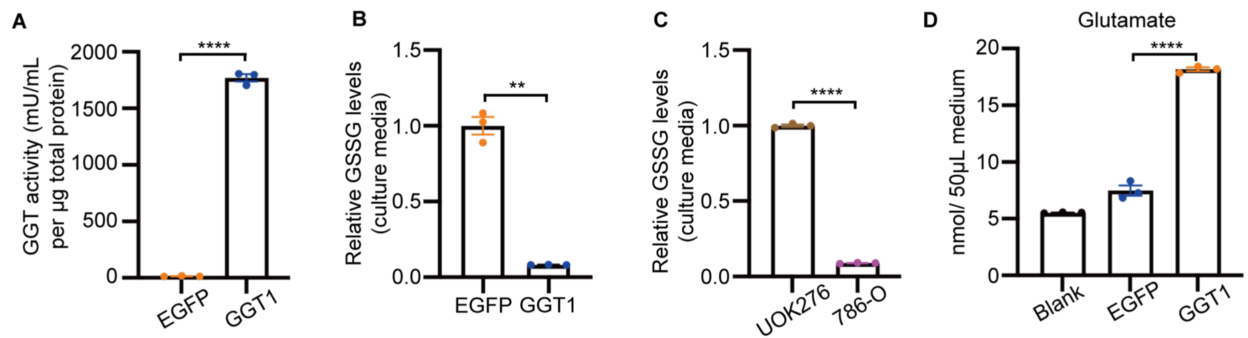

Figure S4. Low GGT1 activity leads to accumulation of extracellular glutathione. (**A**) GGT activity in UOK276 cells with EGFP or GGT1 expression; (**B**) GSSG concentration in the culture media of UOK276 cells with EGFP or GGT1 expression (n=3 biological replicates per condition). (**C**) GSSG concentration in the culture media of UOK276 and 786-O cells (n=3 biological replicates per condition). **(D)** Extracellular glutamate in the medium of UOK276 cells with EGFP or GGT1 expression (n=3 biological replicates per condition). Error bars represent mean ± SD. Statistical significance was calculated using one way ANOVA with Tukey's m2ultiple comparisons test. **$P < 0.01$; ****$P < 0.0001$.

EXTENDED METHODS

Antibodies, drugs, and reagents

The following antibodies were used: GGT1 (catalog # ab109427, Abcam), GPX4 (catalog # ab125066, Abcam), GCLC (catalog # ab190685, Abcam), xCT (SLC7A11, catalog # ab37185, Abcam), NRF2 (catalog # ab76026, Abcam), CREB (catalog # 9197, Cell Signaling Technology), TUBULIN (catalog # 5568, Cell Signaling Technology) and actin (catalog # A5316 Sigma-Aldrich). Imidazole ketone erastin (IKE, catalog # 1801530-11-9), N-acetyl cysteine (catalog # A7250), Erastin (catalog # E7781), 1s,3R-RSL3 (catalog # SML2234), tertiary-butylhydroperoxide (catalog # 416665), glutathione (catalog # 1294820), and ferrostatin-1 (catalog # SML0583) were purchased from Sigma-Aldrich. Cyst(e)inase was supplied by Everett Stone and George Georgiou (University of Texas at Austin).

Crystal violet assay

$1X10^4$ cells per well were seeded in 96-well plates and allowed to attach overnight. Drug treatments were performed on the following day. Following 48 hours of treatment, cells were fixed with 10% formalin for 10 minutes, stained with 0.05% (w/v) crystal violet in distilled water for 20 minutes, washed three times by submerging the plates in clean tap water and air dried. Crystal violet was solubilized by adding 200μL of methanol per well. The absorbance was measured with a Synergy HT plate reader at O.D. 540 (BioTek, Winooski, VT, USA).

Glutathione concentration and mitochondrial GSH/GSSG ratio measurement

Glutathione concentration and the mitochondrial GSH/GSSG ratio were measured using Promega GSH/GSSG-Glo™ Assay kit according to the manufacturer's recommended protocol. Briefly, $2X10^4$ cells per well were seeded in 96-well white opaque plates and were allowed to attach overnight. The medium was removed and replaced with the provided lysis reagent. Luciferin generation reagent was added to all wells, and assays were mixed and incubated for 30 minutes. Luciferin detection reagent was added to all wells, assays were mixed, and after a 15-minutes incubation, luminescence was measured on Synergy HT plate reader (BioTek, Winooski, VT, USA).

mRNA Expression Analysis

Two micrograms of total RNA (RNeasy MicroKit; Qiagen Inc.) were retrotranscribed with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Five nanograms of cDNA per reaction were run using TaqMan probes as following (Thermofisher).

Taqman probers	Catalog number
GGT1	Hs00980756_m1
GCLC	Hs00155249_m1
GPX4	Hs00989766_g1
KEAP1	Hs00202227_m1
SLC7A11	Hs00921935_m1

Reactive oxygen species measurements

ROS levels were measured using H2DCFDA (catalog # ab113851, Abcam) and Deep Red assay kit (catalog # ab186029, Abcam), as described previously (74). Cells were washed once with PBS (Phosphate buffered saline, Gibco, 10010-023) and incubated with 10 µM H2DCFDA PBS for 30 minutes at 37°C. Fluorescent intensity for H2DCFDA

(Ex/Em: 492/520 nm) was determined by flow cytometry (BD FACS Canto II, BD Biosciences), and analyzed with FlowJo analysis software (Treestar).

Lipid peroxidation analysis by flow cytometry

For flow cytometry, 50000 cells were seeded in 6-well plate in the appropriate cell culture media, or supplemented with 600 nM of IKE where indicated, cultured overnight, and incubated with 5 μM of BODIPY C11 dye for the last 45 minutes. Before flow cytometry, cells were washed with PBS twice, trypsinized and filtered into single-cell suspensions. Fluorescence was assessed by flow cytometry (BD FACS Canto II, BD Biosciences), and analyzed with FlowJo analytical software (Treestar). Median fluorescence intensity of FITC was measured in each sample.

Cystine-FITC assay by flow cytometry

50000 cells were seeded in 6-well plates, and supplemented with 5 μM cystine-FICT (Sigma, SCT047) and/or 1 μM of IKE as indicated. Before flow cytometry, cells were washed with PBS twice, trypsinized and filtered into single-cell suspensions. Fluorescence was assessed by flow cytometry (BD FACS Canto II, BD Biosciences) and analyzed with FlowJo analytical software (Treestar). Median fluorescence intensity of FITC was measured in each sample.