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Supplemental Information

A Genome-wide Haploid Genetic Screen Identifies Regulators of Glutathione Abundance and Ferroptosis Sensitivity

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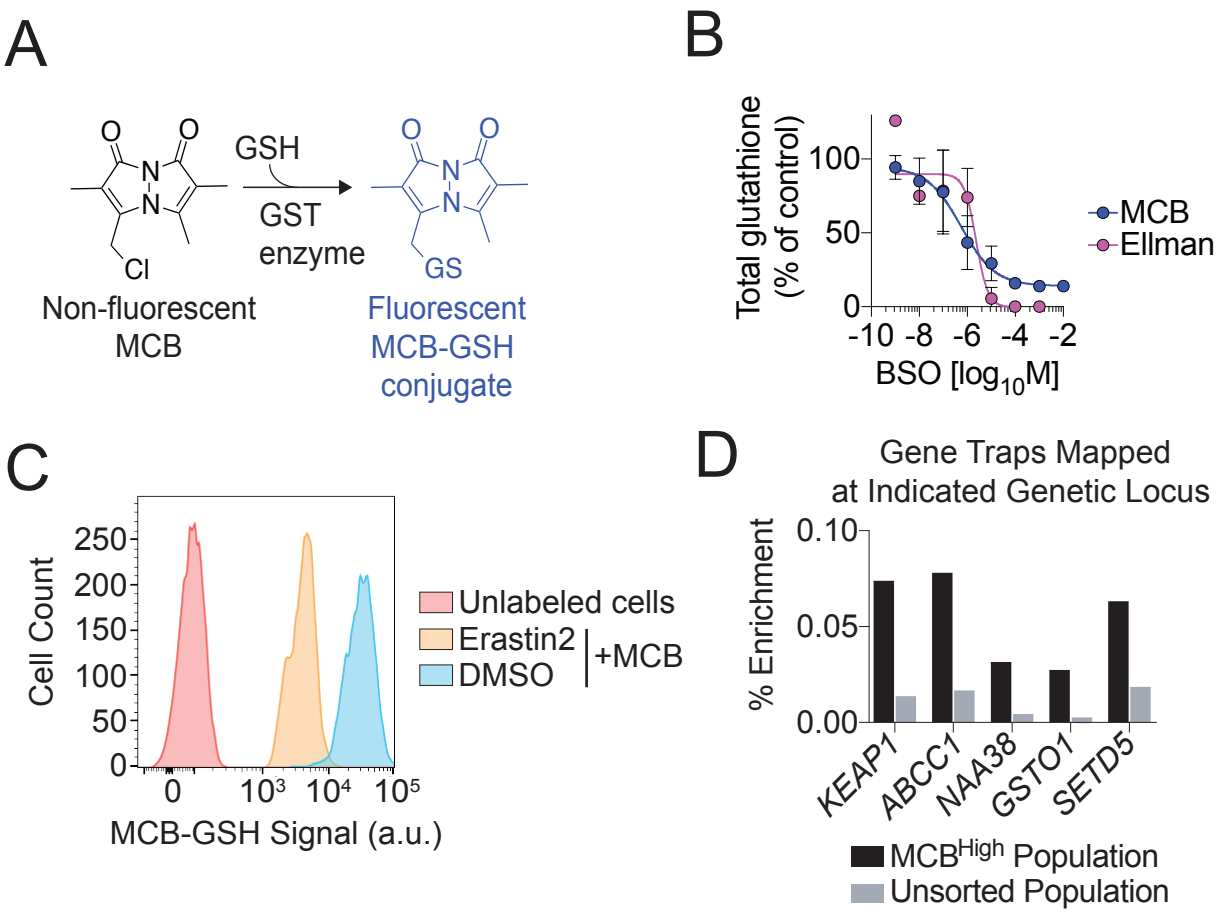
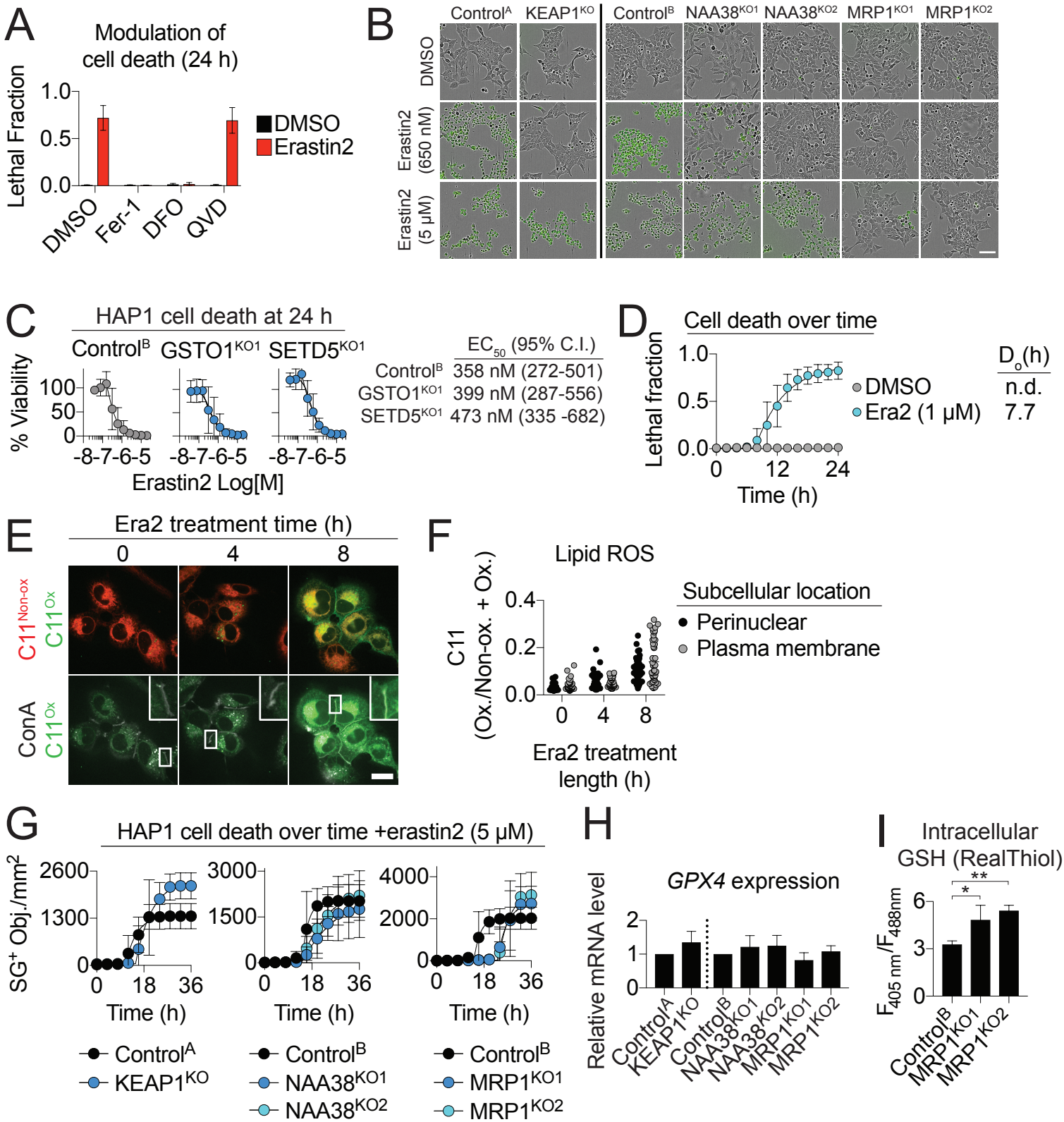
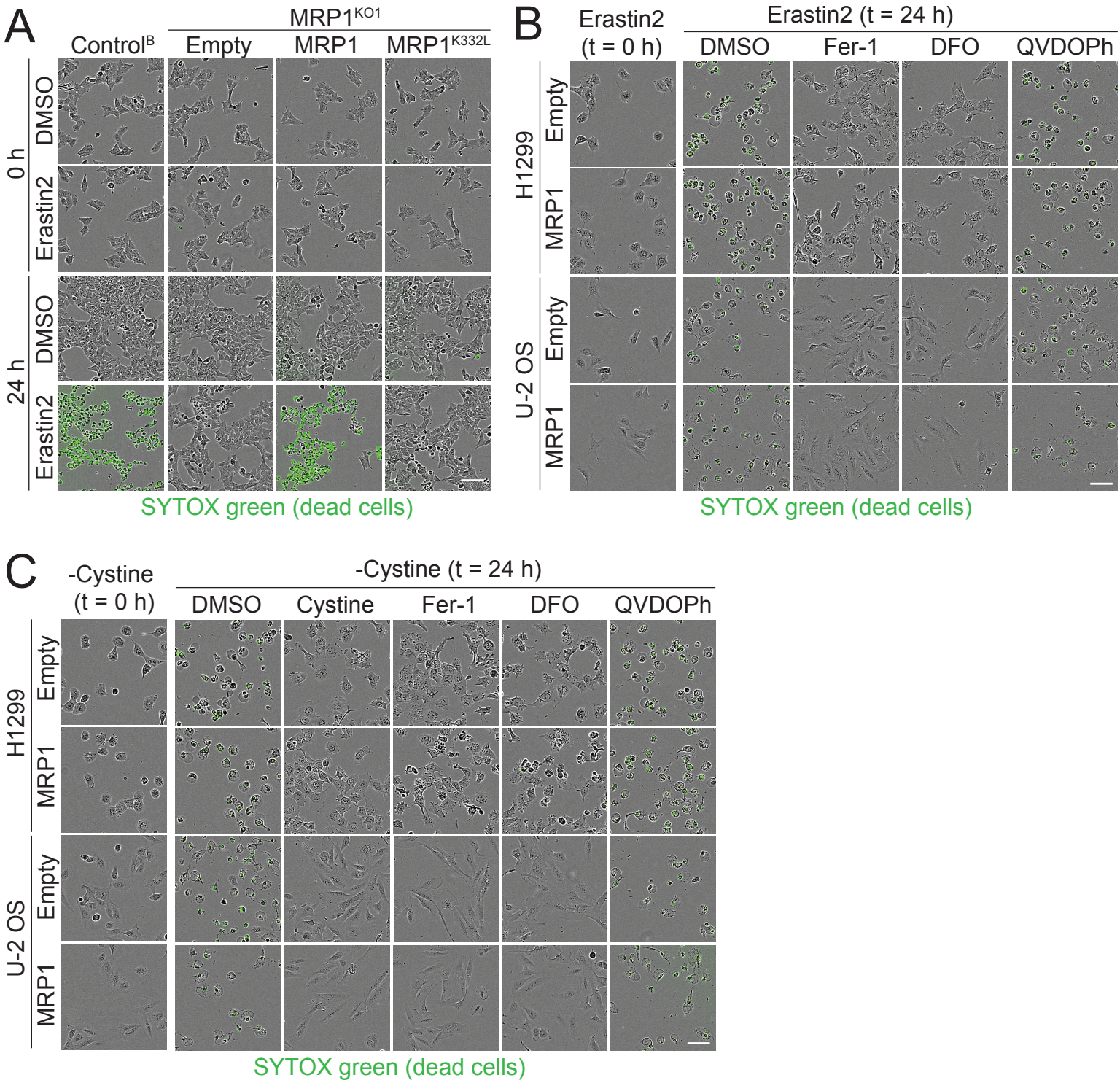


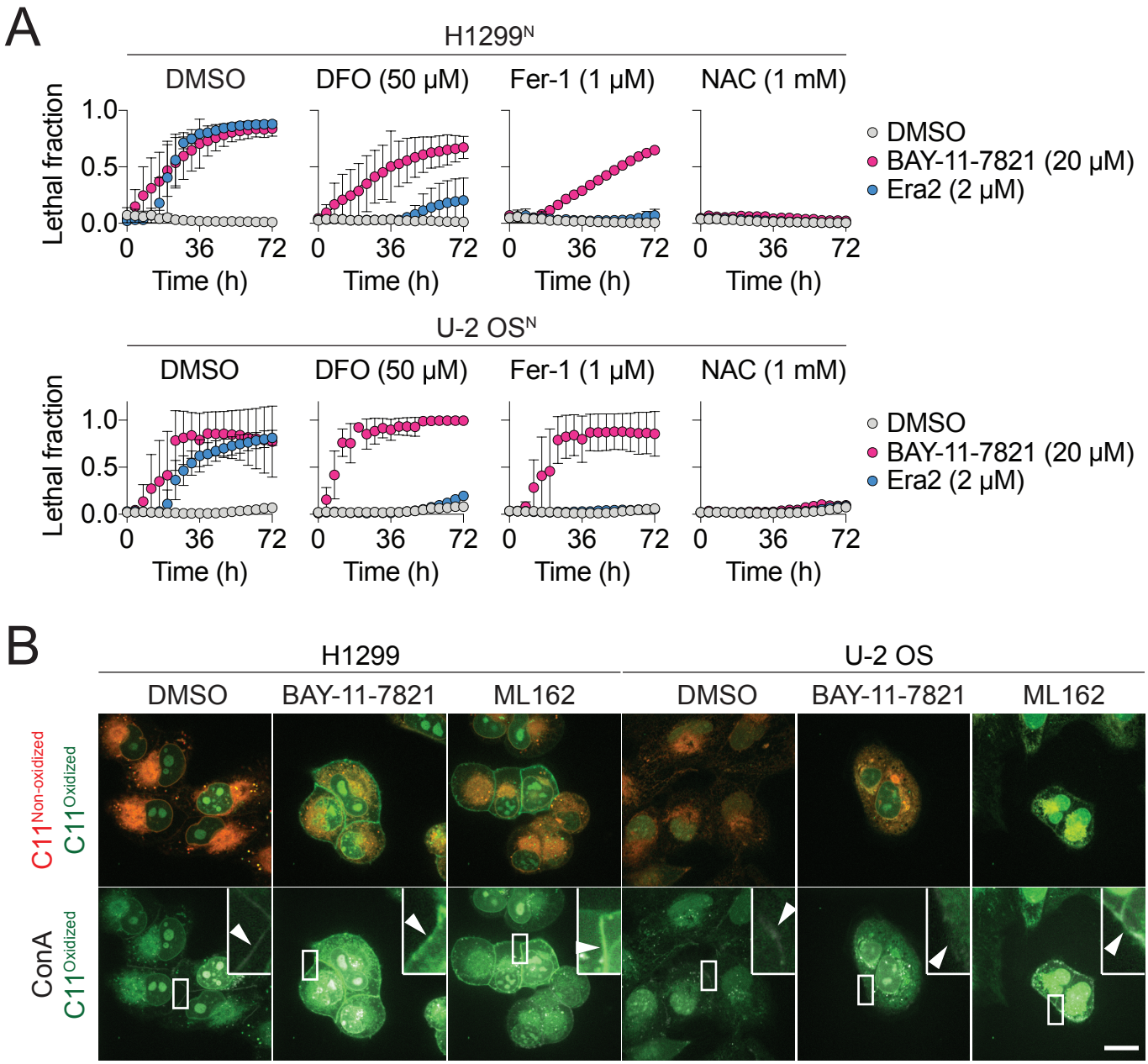
Supplemental Figure 1, related to Figure 1. A haploid screen for regulators of glutathione abundance. (A) Chemical structure of monochlorobimane (MCB), a probe whose fluorescence is increased by conjugation to reduced glutathione (GSH) by glutathione-S-transferase (GST) enzymes. (B) Intracellular total glutathione detected in HAP1 cells using MCB (via flow cytometry) and Ellman's reagent (via biochemical assay) in response to the GSH biosynthesis inhibitor, buthionine sulfoxomine (BSO, 24 h). Data represent mean ± SD from three independent experiments. (C) Flow cytometry data of MCB-labeled HAP1 cells in response to 24 h treatment ± erastin2 (5 μM) + ferrostatin-1 (Fer-1, 2 μM). Fer-1 was included to prevent cell death at this timepoint prior to GSH analysis. a.u.: arbitrary units. 50,000 cells were counted per condition. (D) Percent enrichment of gene trap insertions mapped to the indicated gene loci relative to total gene traps sequenced in the isolated 'MCB^{High}' population versus unsorted cells.



Supplemental Figure 2, related to Figure 2. Regulation of ferroptosis sensitivity by MRP1 and other genes. (A) HAP1^{N} cells treated with DMSO or erastin2 (5 µM) ± ferrostatin-1 (Fer1, 2 µM), deferoxamine (DFO, 100 µM) and Q-VD-OPh (QVD, 25 µM) for 24 h. Cell death was assayed using STACK. (B) Representative images of Control$^{A/B}$, NAA38$^{KO1/2}$ and MRP1$^{KO1/2}$ ± erastin2 treatment at 24 h. Dead cells are positive for SYTOX Green (20 nM), included in the growth medium of all cells. Scale bar = 50 µm. (C) Cell viability data in HAP1 Control^{B}, GSTO^{KO1} and SETD5^{KO1} cells assayed by PrestoBlue. Mean and 95% C.I. of erastin2 EC_{50} are shown. Note: the viability data for Control^{B} cells is same as shown in Figure 1C. (D) Cell death over time in HT-1080^{N} cells. Era2: erastin2, n.d. = not determinable. (E) C11 BODIPY 581/591 (C11) confocal imaging of HT-1080 cells. Cells were treated with Era2 (1 µM) for the indicated times, then labeled with Concanavalin A-Alexa Fluor 350 (ConA), which labels the plasma membrane, and C11. Non-ox: non-oxidized, Ox: oxidized. Scale bar = 20 µm. (F) Quantification of perinuclear and plasma membrane C11. Each dot represents an individual cell. (G) Cell death over time of Control$^{A/B}$, KEAP1^{KO}, NAA38$^{KO1/2}$ and MRP1$^{KO1/2}$ cells treated with erastin2 (5 µM). (H) (E) Relative GPX4 mRNA expression determined using RT-qPCR. (I) Intracellular GSH assayed in Control^{B} and MRP1$^{KO1/2}$ cells assessed using RealThiol (1 µM). *P < 0.05, *P < 0.01, one-way ANOVA. Data in A,C,D and G-I represent mean ± SD from three independent experiments. Imaging (E) and quantification (F) were performed on two independent biological replicates, and results from one replicate are shown.



Supplemental Figure 3, related to Figure 2 and Figure 3. (A) Images of HAP1 control or MRP1^{KO1} cells stably complemented with wildtype MRP1, a GSH-export defective mutant MRP1^{K332L} or vector control (Empty), treated with DMSO or erastin2 (5 μM) at the start of the experiment (t = 0 h) or after 24 h of treatment. Dead cells are observed by the uptake of SYTOX Green dye. (B,C) Images aquired at the start of the experiment (t = 0 h) or after 24 h of treatment for H1299 or U-2 OS cells transduced with a control (Empty) vector or a MRP1 overexpression vector, and treated with erastin2 (1 μM, B) or switched to medium lacking cystine (-cystine, C) ± ferrostatin-1 (Fer1, 2 μM), deferoxamine (DFO, 100 μM) or Q-VD-OPh (QVD, 25 μM). All panels are representative images from one of three independent experiments and dead cells are marked by uptake of SYTOX Green. Scale bar = 50 μm.



Supplemental Figure 4, related to Figure 4. Investigating the lethal effects of BAY compounds. (A) Cell death determined over time using STACK in response to the indicated lethal compounds and inhibitors. Data represent mean ± SD from three independent experiments. (B) Confocal imaging of lipid ROS in HT-1080 cells. Cells were treated with DMSO (vehicle), BAY-11-7821 (20 μM) or ML162 (5 μM) for 4 h, then incubated with C11 BODIPY 581/591 (C11), which detects lipid ROS, and concanavalin A-Alexa Fluor 350 (ConA), which labels the plasma membrane. Non-ox: non-oxidized, Ox: oxidized. Arrowheads within the insets indicate regions of high or low C11 oxidation at the plasma membrane. Data are representative of two independent experiments. Scale bar = 20 μm.

Cell Type	Gene Edited	Cell Line	Sequence	Editing Event
HAP1	*MRP1*	Control[B]	5'-AAGTGCTTTCAGAACACGGTCCTCGTGTGGGTGCCTTG-3'	-
		MRP1^{KO1}	5'-AAGTGCTTTCAGAACACA-----GTGTGGGTGCCTTG-3'	5 bp deletion, 1 bp insertion
		MRP1^{KO2}	5'-AAGTGCTTTCAGAACACGGACCG----------TG-3'	13 bp deletion, 1 bp insertion, 1 bp alteration
	NAA38	Control[B]	5'-CATTCCCCGGGCACTGACCCGACGGCTTGAGGAACTCC -3'	-
		NAA38^{KO1}	5'-CATTCCCCGGGCACTGA----------GGAACTCC -3'	13 bp deletion
		NAA38^{KO2}	5'-CATT----------CTCC -3'	31 bp deletion
	GSTO1	Control[B]	5'-GCTGTAGATGCGGATCGAGCCCTCCGGGACCGGCCCGG-3'	-
		GSTO1^{KO1}	5'-GCTGTAGATGCGGATCGAGCCC----------CGG-3'	13 bp deletion
		GSTO1^{KO2}	5'-GCTGTAGATGCGGATCGAGCCCCTCCGGGACCGGCCCGG-3'	1 bp insertion
	SETD5	Control[B]	5'-CCCTGTCGATCATCCCTCGTTCTGACCTGAAT -3'	-
		SETD5^{KO1}	5'-CCCTGT-(176 bp)-GATCAT -3'	176 bp deletion
		SETD5^{KO2}	5'-CCCTGTCCAGACGATCATCCCTCGTTCTGACCTGAAT -3'	5 bp insertion
A549	*MRP1*	Control	5'-AAGTGCTTTCAGAACACGGTCCTCGTGTGGGTGCCTTG-3'	-
		MRP1^{KO1}	5'-AAGTGCTTTCAGAACACGGTCCTC-TGTGGGTGCCTTG-3'	1 bp deletion
		MRP1^{KO2}	5'-AAGTGCTTTCAGAACACGGTCCTCG----GGTGCCTTG-3'	4 bp deletion

Table S1, Related to STAR Methods. Genotyping of control and mutant cell lines. CRISPR/Cas9 guide sequences are in red. Deletions are denoted by "–" ; insertions are in blue; alterations are in green.

OLIGONUCLEOTIDE NAME AND SEQUENCE	SOURCE	IDENTIFIER
sgRNA for *ABCC1/*MRP1 [5'-TTCAGAACACGGTCCTCGTG-3']	Designed using crispr.mit.edu	N/A
sgRNA for *NAA38* [5'-GGCACTGACCCGACGGCTTG-3']	Designed using crispr.mit.edu	N/A
sgRNA for *GSTO* [5'-TAGATGCGGATCGAGCCCTC-3']	Designed using crispr.mit.edu	N/A
sgRNA for *SETD5* [5'-CAGAACGAGGGATGATCGTC-3']	Designed using crispr.mit.edu	N/A
Forward primer for sequencing *ABCC1/*MRP1 gene locus [5'-GCCTTGTCTGTTTCTTC-3']	This paper	N/A
Reverse primer for sequencing *ABCC1/*MRP1 gene locus [5'- CATGCTCCAGGCGAGC-3']	This paper	N/A
Forward primer for sequencing *NAA38* gene locus [5'-GCATCCCAGCTACACACAGA-3']	This paper	N/A
Reverse primer for sequencing *NAA38* gene locus [5'-GTACGCTTCAGTGAGCCACA-3']	This paper	N/A
Forward primer for sequencing *SETD5* gene locus [5'-TATGGGACCACTCAGAGGCA-3']	This paper	N/A
Reverse primer for sequencing *SETD5* gene locus [5'-ACATGGGCGAAGTGTCTCTG-3']	This paper	N/A
Forward primer for sequencing *GSTO* gene locus [5'-TCCTGAATCCCCTGCAAACC-3']	This paper	N/A
Reverse primer for sequencing *GSTO* gene locus [5'-AACCAACAGCCCAATCCACA-3']	This paper	N/A
Human *ACTB* qPCR forward primer [5'-ATCCGCCGCCCGTCCACA-3']	Tarangelo et al., 2018	N/A
Human *ACTB* qPCR reverse primer [5'-ACCATCACGCCCTGGTGCCT-3']	Tarangelo et al., 2018	N/A
Human *SLC7A11* qPCR forward primer [5'-GGGCATGTCTCTGACCATCT-3']	Tarangelo et al., 2018	N/A
Human *SLC7A11* qPCR reverse primer [5'-TCCCAATTCAGCATAAGACAAA-3']	Tarangelo et al., 2018	N/A
Human *GCLM* qPCR forward primer [5'-CATTTACAGCCTTACTGGGAGG-3']	Tarangelo et al., 2018	Accession NM_002061.3
Human *GCLM* qPCR reverse primer [5'-ATGCAGTCAAATCTGGTGGCA-3']	Tarangelo et al., 2018	N/A
Human *GCLC* qPCR forward primer [5'-GGCGATGAGGTGGAATACAT-3']	Tarangelo et al., 2018	N/A
Human *GCLC* qPCR reverse primer [5'-GTCCTTTCCCCCTTCTCTTG-3']	Tarangelo et al., 2018	N/A
Human *NQO1* qPCR forward primer [5'-GCCGCAGACCTTGTGATATT-3']	Tarangelo et al., 2018	N/A
Human *NQO1* qPCR reverse primer [5'-TTTCAGAATGGCAGGGACTC-3']	Tarangelo et al., 2018	N/A
Human *NRF2* qPCR forward primer [5'-GAGAGCCCAGTCTTCATTGC-3']	Tarangelo et al., 2018	N/A
Human *NRF2* qPCR reverse primer [5'-TGCTCAATGTCCTGTTGCAT-3']	Tarangelo et al., 2018	N/A
Human *GPX4* qPCR forward primer [5'-AGACCGAAGTAAACTACACTCAGC-3']	Gautrey et al., 2011	N/A

Human *GPX4* qPCR reverse primer [5'-CGGCGAACTCTTTGATCTCT-3']	Gautrey et al., 2011	N/A

Table S2, Related to STAR Methods. Oligonucleotides used in this study.