Supporting Information

A Compendium of Kinetic Modulatory Profiles Identifies Ferroptosis Regulators

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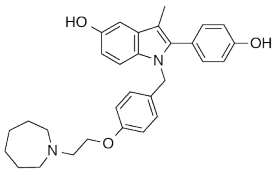
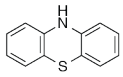
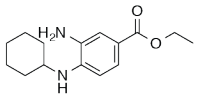
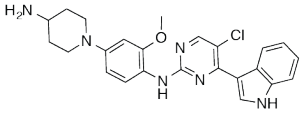
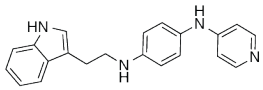
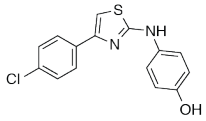
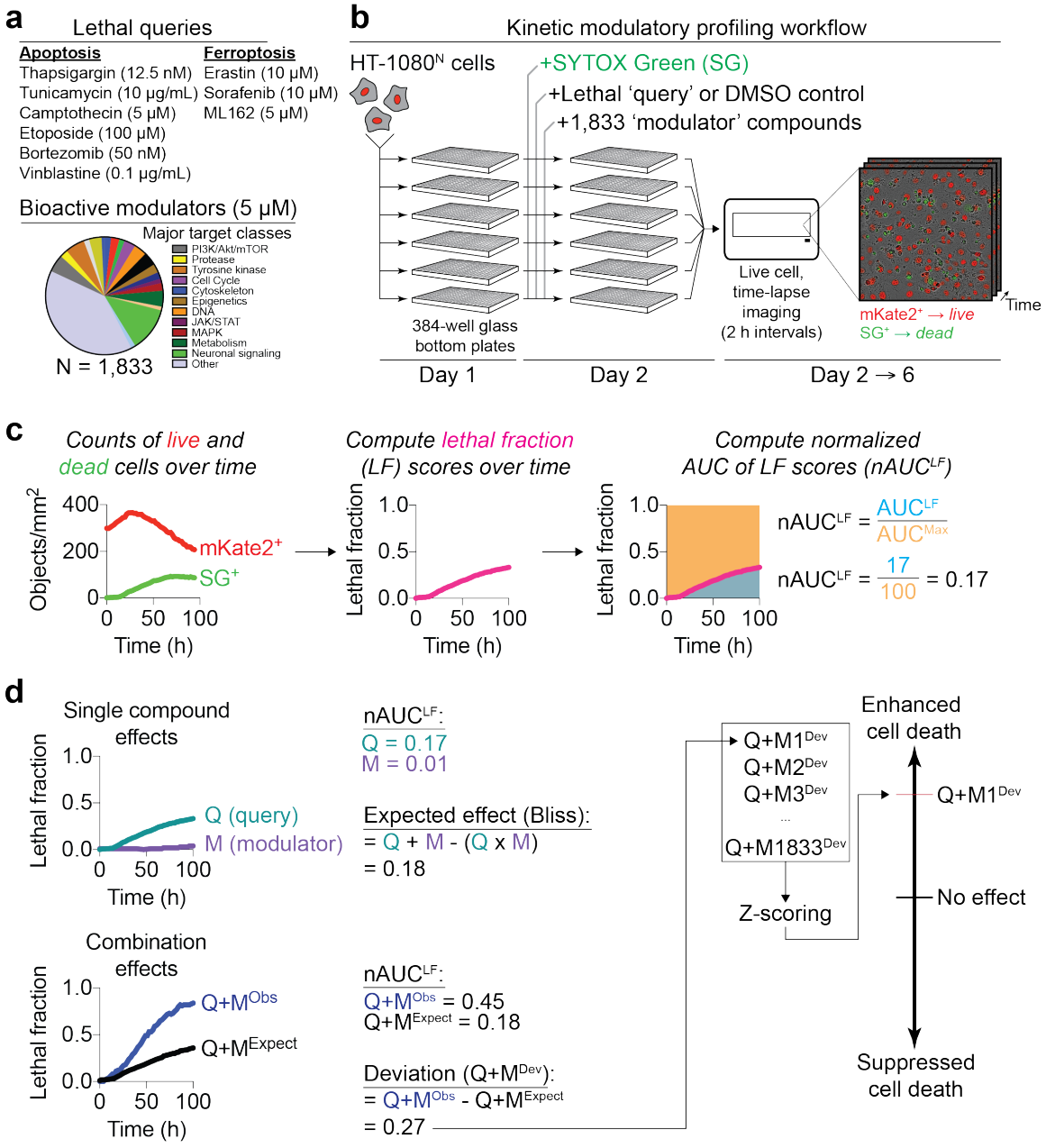
This file contains Supplementary Table 1, Supplementary Figures 1-5, and Supplementary References.

Supporting Tables

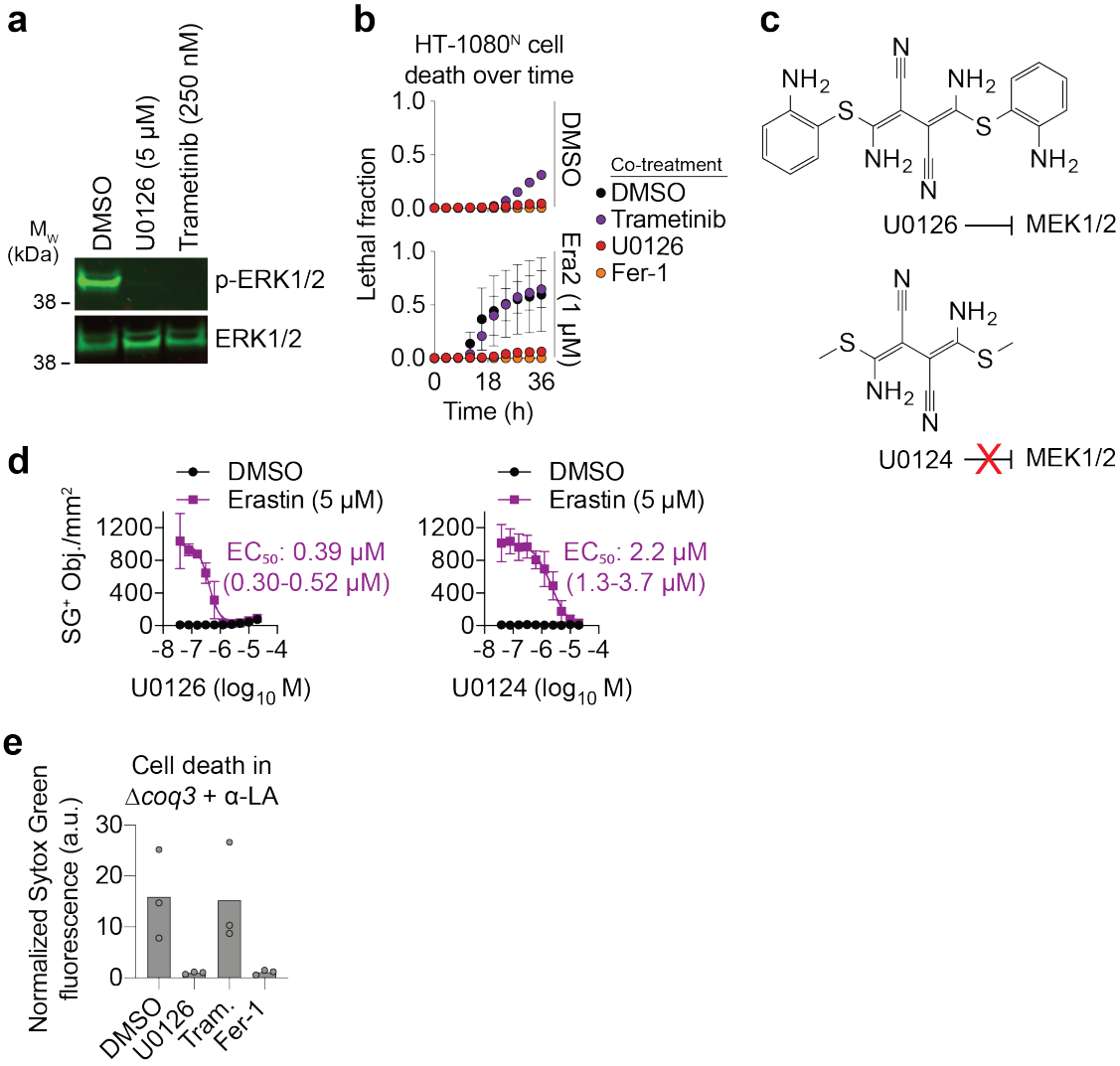
Compound		Ferroptosis suppression EC_{50} [95% C.I] nM	
Name	Structure	1 μM Erastin2	2 μM ML162
Bazedoxifene		48 (41 - 56)	15 (ND - 34)
Phenothiazine		50 (41 - 61)	4.4 (2.3 - 5.9)
Fer-1		52 (49 - 62)	10 (ND - 28)
AZD3463		71 (60 - 83)	13 (ND - 25)
Serdemetan		82 (52 - 350)	12 (6.6 - 19)
SKI II		106 (97 - 115)	12 (ND - 24)

Supplementary Table 1. Dose-response testing of ferroptosis inhibitors identified as DPPH free radical scavengers. Name, structure and inhibitor potency in cell-based assays. HT-1080^{N} cells were treated with lethal compounds (erastin2, ML162 or H_2O_2) at a fixed concentration and cotreated with a ten-point, two-fold concentration-response series of each inhibitor, starting at 10 μM. Lethal fraction data derived from STACK analysis at the 24 h timepoint were used to compute EC_{50} values. Data are mean ± 95% confidence interval (95% C.I.) from three independent experiments. ND: not defined.

Supplementary Figures

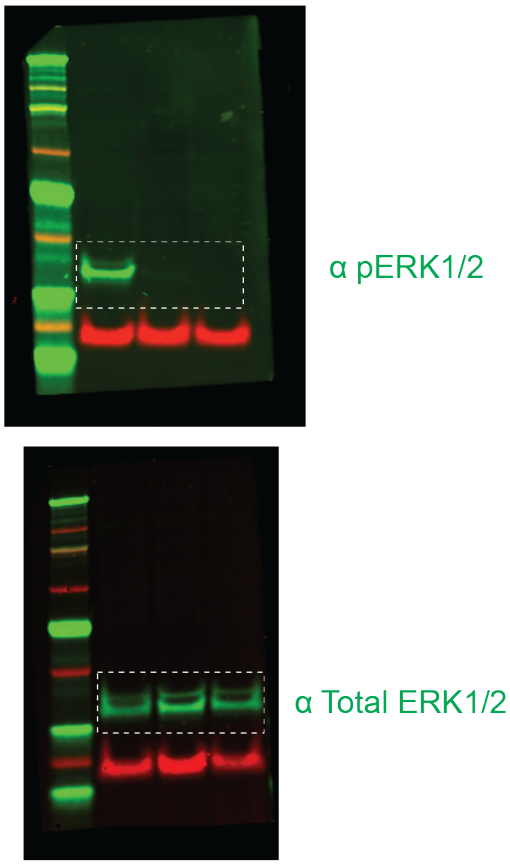


Supplementary Figure 1. Overview of kinetic modulatory profiling. a, Summary of lethal molecules and query molecules used in the kinetic modulatory profiling. **b**, Illustration of the kinetic modulatory workflow. **c**, Calculation of normalized area under the curve of lethal fraction (LF) scores ($nAUC^{LF}$). **d**, Illustration of the calculation of modulatory effects using $nAUC^{LF}$ scores. The Bliss model of drug interaction was employed to compute the expected compound combination effects.

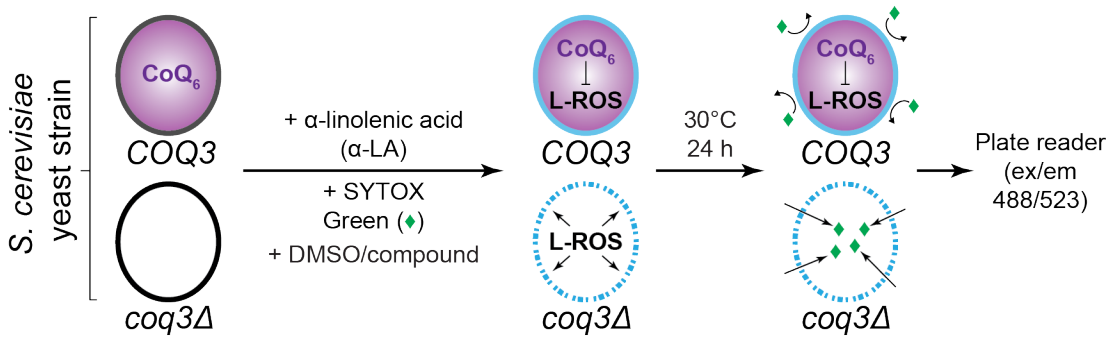


Supplementary Figure 2. U0126 can act as an antioxidant suppressor of ferroptosis. **a**, Western blot of serum starved HT-1080 cells stimulated with media containing 10% FBS for 30 min ± DMSO (vehicle), U0126 (5 µM) or trametinib (250 nM), a potent allosteric MEK1/2 inhibitor. Blots were probed for phospho-p44/42 MAPK (ERK1/2) and total MAPK (ERK1/2). One representative blot from three independent experiments is shown. **b**, Cell death over time. Compound concentrations are as in (a), with ferrostatin-1 (Fer-1, 1 µM). **c**, Structure of U0126 and the analog U0124. U0126 inhibits mitogen activated protein kinase kinase 1/2 (MEK1/2), while U0124 does not[1]. **d**, SYTOX Green positive (SG^+) dead cells at 24 h. Like U0126, U0124 can also inhibit erastin-induced ferroptosis. **e**, U0126 inhibits cell death in a yeast assay for polyunsaturated fatty acid autoxidation-induced cell death, while trametinib (Tram.) does not. α-LA: alpha-linolenic acid. All inhibitors were tested at 5 µM. Results in **b** and **d** represent mean ± SD from three independent experiments, while data in **e** is from three independent experiments.

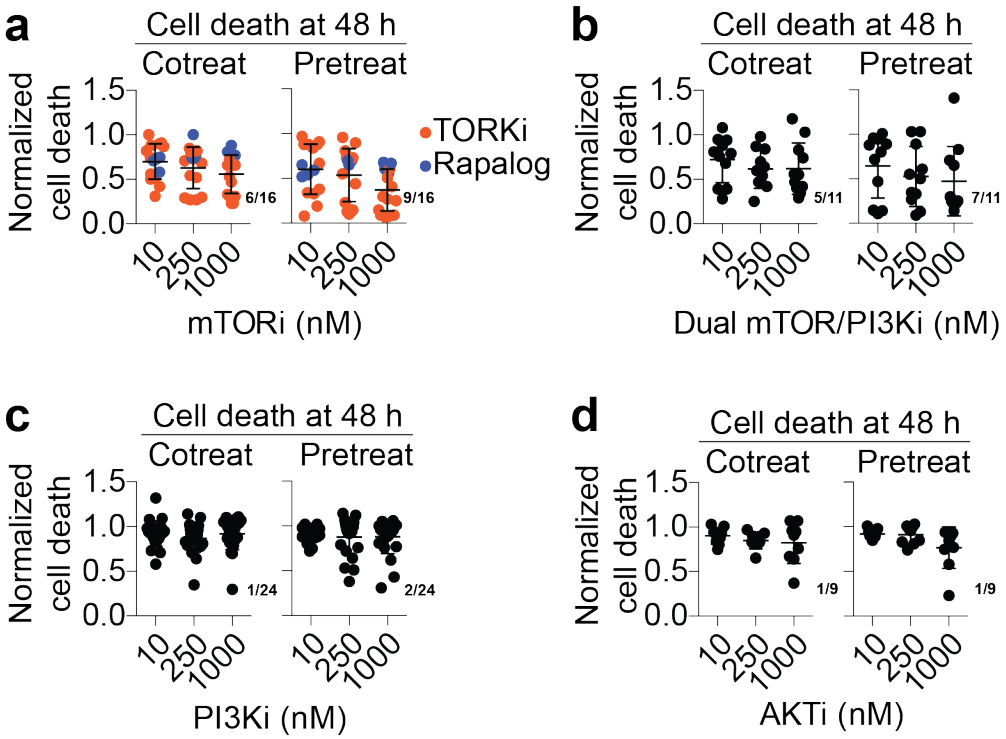
Supplementary Fig. 2a



Supplementary Figure 3. Source data for Supplementary Figure 2.



Supplementary Figure 4. Schematic of a *Saccharomyces cerevisiae* (*S. cerevisiae*)-based ferroptosis assay. The polyunsaturated fatty acid α-linolenic acid (α-LA) is oxidized to form lipid reactive oxygen species (L-ROS). COQ3 (i.e. wild-type) yeast produce Coenzyme Q6 (CoQ6) to detoxify L-ROS and prevent damage to the plasma membrane (solid blue line). CoQ6-defective *coq3Δ* yeast cannot detoxify L-ROS, resulting in a permeabilized (i.e., damaged) plasma membrane (dashed blue line). In the assay, *coq3Δ* yeast are cotreated with ethanol (EtOH) or α-LA (500 μM), the dead cell marker SYTOX Green (250 nM), and DMSO or the candidate antioxidant or iron chelator for 24 h. At the end of the incubation, cell death is measured by determining SYTOX Green uptake (excitation at 488 nm/emission at 523 nm).



Supplementary Figure 5. mTOR regulates ferroptosis. a-d, Cell death measured in U-2 OS cells by imaging of SYTOX Green positive (SG^+) dead cells at 48 h. Cells were treated with erastin (20 μM). Cell death for each condition is normalized to erastin-only controls (0 = alive, 1 = dead). Cells were either cotreated (Cotreat) or pretreated for 6 h (Pretreat) with small molecule inhibitors targeting mTOR (n = 16, **a**), mTOR/PI3K (n = 11, **b**), PI3K (n = 24, **c**) or AKT (n = 9, **d**). Each inhibitor was tested at 1000 nM, 250 nM or 10 nM. Each dot represents a single inhibitor tested in one experiment. In **a**, ATP competitive mTOR inhibitors (n = 12) are colored orange and rapalogs (n = 4) are colored blue. The number of inhibitors in each class that suppress cell death by at least 50% at 1000 nM are shown beside each graph.

Supplementary References

1 Favata, M. F. *et al.* Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* **273**, 18623-18632, doi:10.1074/jbc.273.29.18623 (1998).