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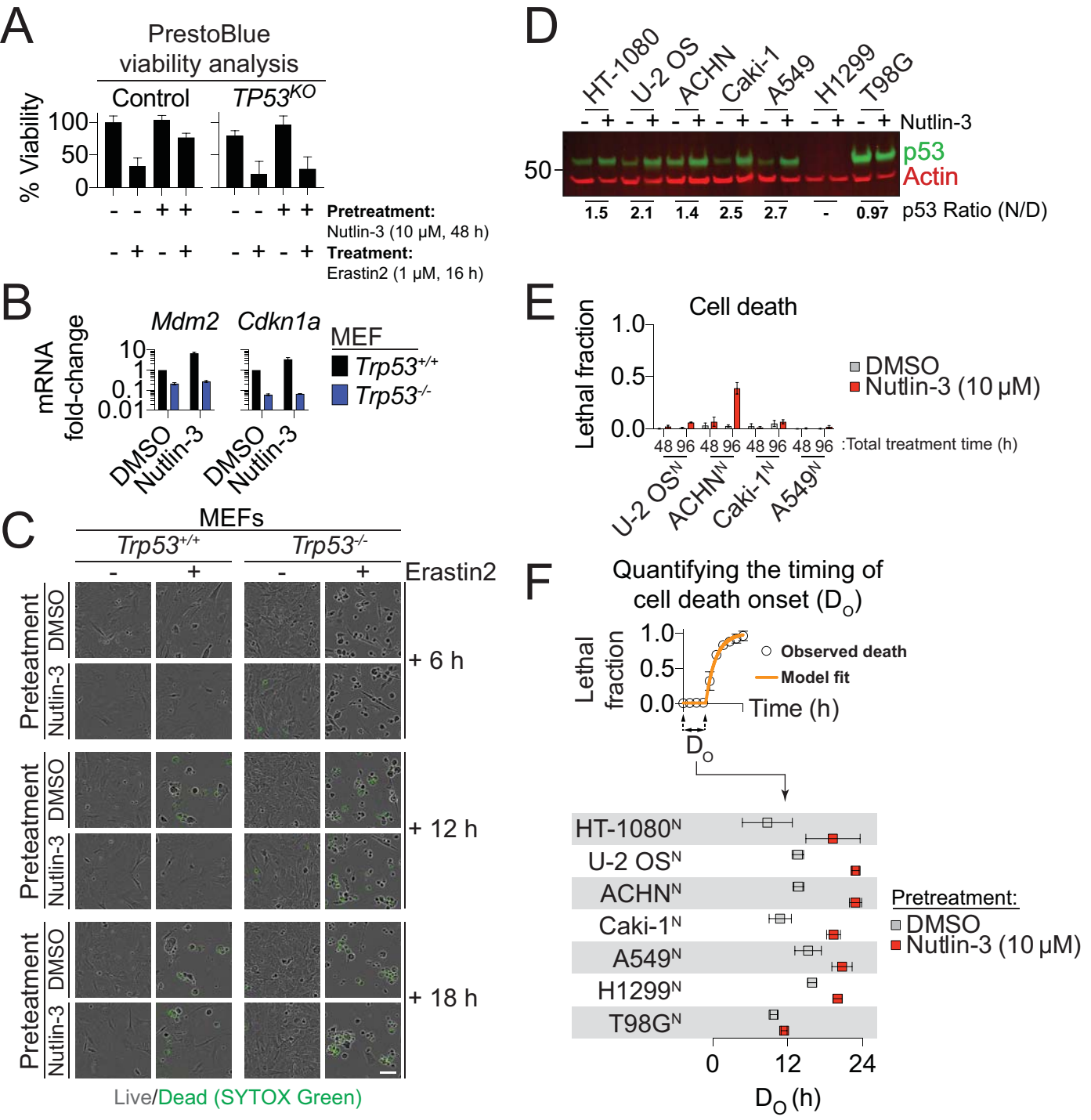
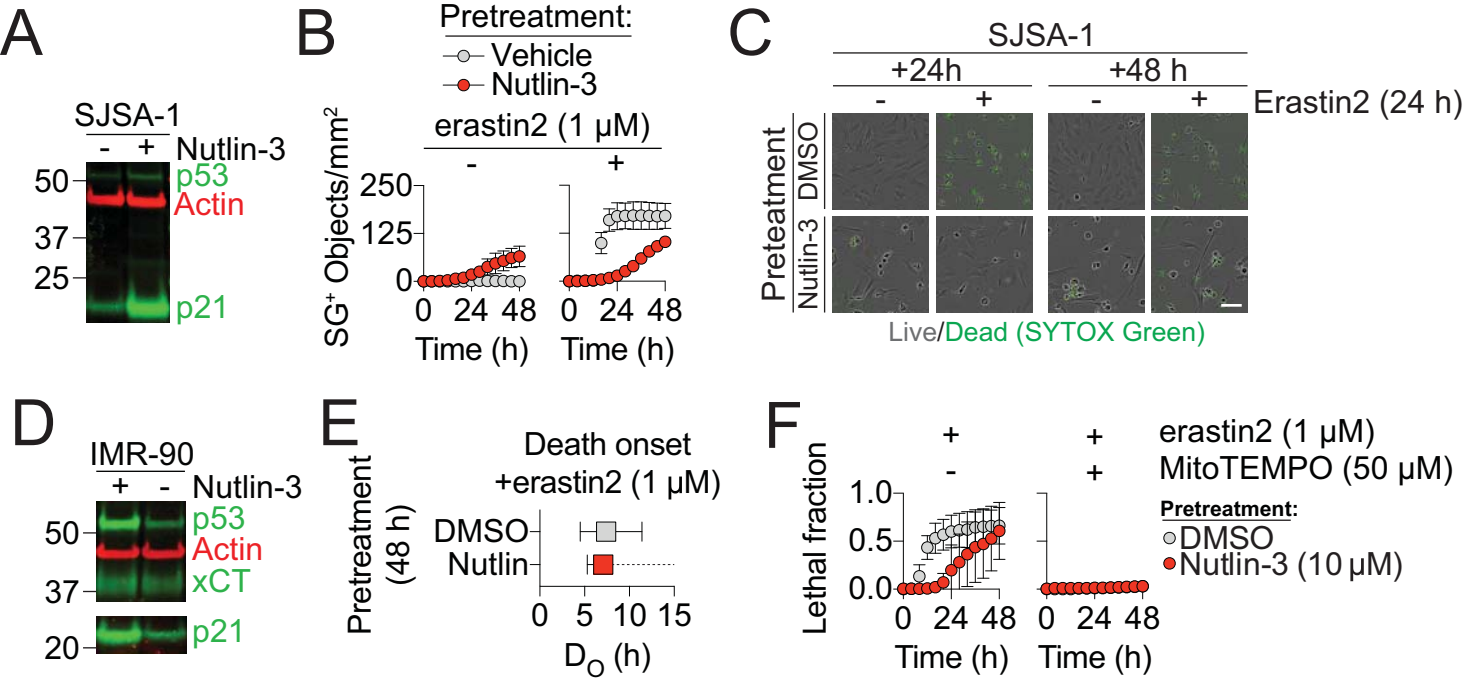
Supplemental Information

p53 Suppresses Metabolic Stress-Induced Ferroptosis in Cancer Cells

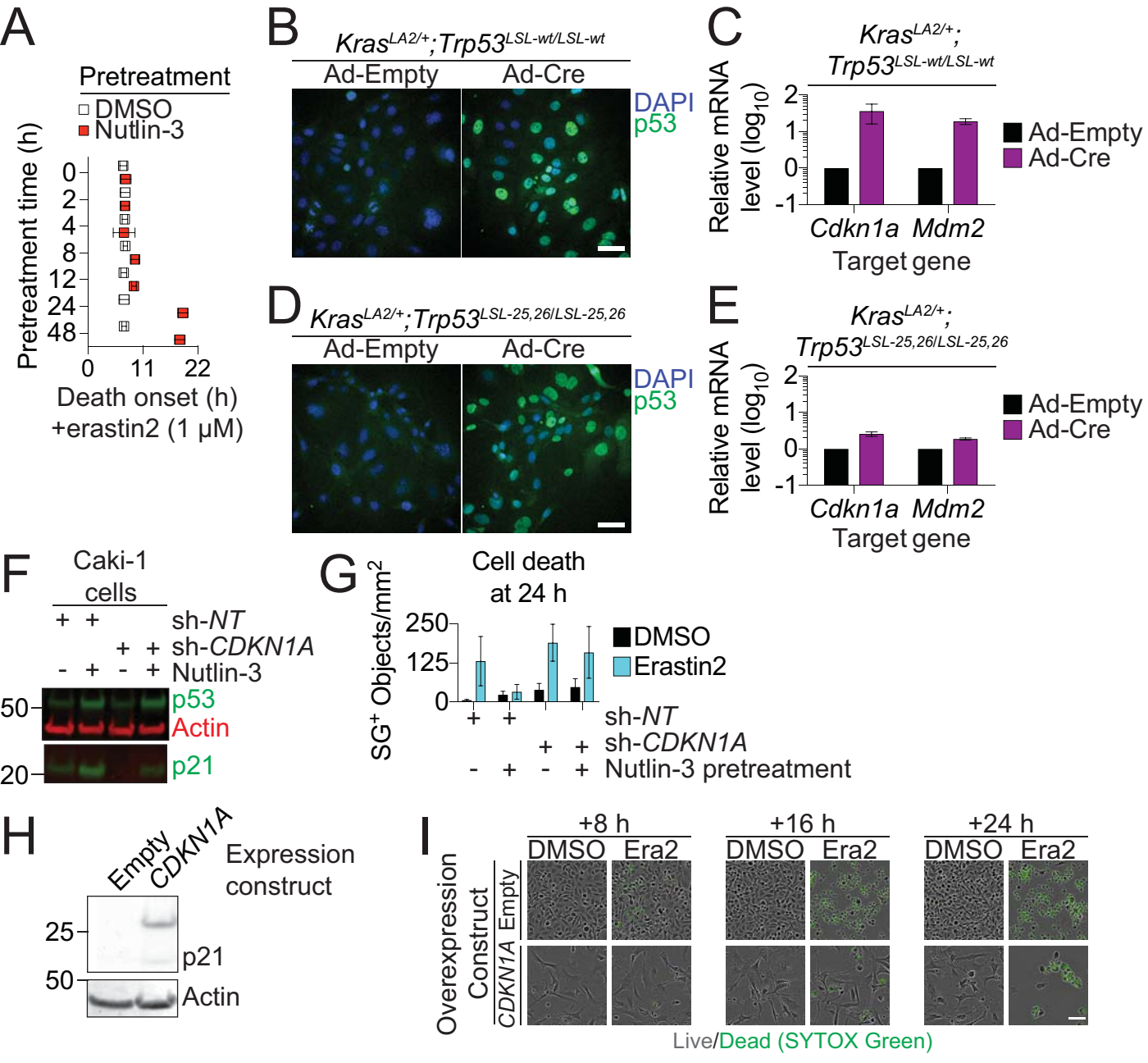
Amy Tarangelo, Leslie Magtanong, Kathryn T. Bieging-Rolett, Yang Li, Jiangbin Ye, Laura D. Attardi, and Scott J. Dixon



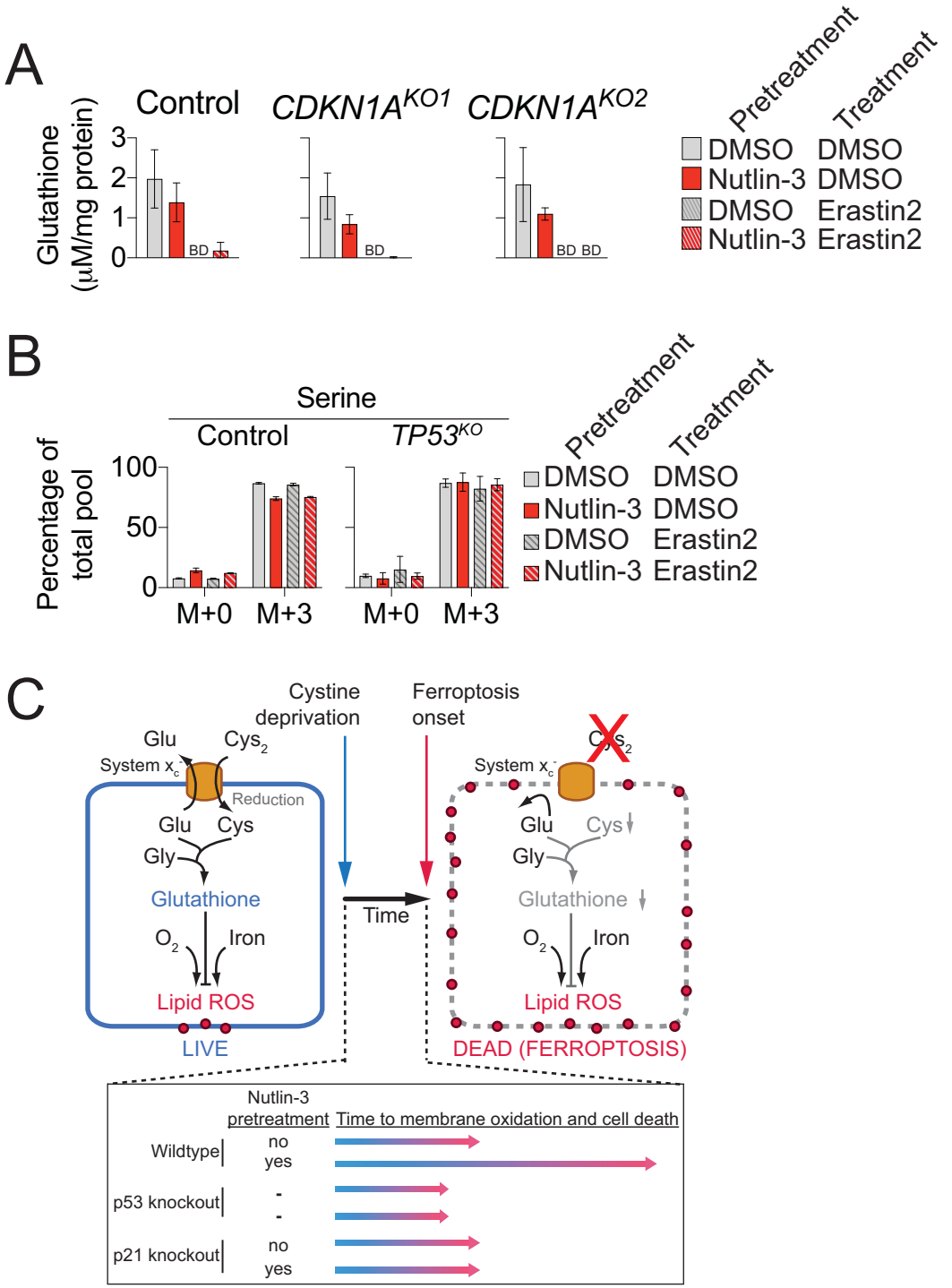
Supplemental Figure 1. p53 stabilization suppresses ferroptosis, Related to Figure 1. (A) Relative HT-1080 cell viability assessed using PrestoBlue at 16 h ± erastin2, following pretreatment ± nutlin-3. (B) Mdm2 and Cdkn1a levels in $p53^{+/+}$ and $p53^{-/-}$ MEFs. (C) Representative images of $Trp53^{+/+}$ and $Trp53^{-/-}$ MEFs pretreated ± nutlin-3 (10 μM, 48 h), then treated ± erastin2 (250 nM) and imaged at 6, 12 or 18 h in the presence of SYTOX Green. Images are representative of three independent experiments. The scale bar = 25 μm. (D) p53 levels ± nutlin-3 (10 μM, 48 h) in a panel of cancer cell lines. The actin-normalized ratio of p53 level in nutlin-3 versus DMSO treatment conditions is indicated. (E) Cell death following 48 h and 96 h ± nutlin-3 (10 μM) in four cell lines. (F) The timing of cell death onset (D_O) within the population can be quantified using STACK. D_O in a panel of cell lines in response to erastin2 (1 μM) ± nutlin-3 (10 μM, 48 h) pretreatment. Data represent mean ± SD from two (B) or three (A,E,F) biological replicates.



Supplemental Figure 2. Effect of p53 stabilization on ferroptosis, Related to Figure 1. (A) p53 and p21 levels in SJSA-1 cells ± nutlin-3 (10 μM, 24 h). (B) Dead cell (SYTOX Green, SG) counts over time in SJSA-1 cells ± erastin2 following pretreatment ± nutlin-3 (10 μM, 24 h). (C) Images from the experiments summarized in B, showing cell death at 24 and 48 h. The scale bar equals 25 μm. Note: the images for DMSO-pretreated, erastin2-treated cells at +24 h and +48 h appear highly similar as cell death is essentially complete by 24 h with little change thereafter. (D) p53, xCT and p21 levels in IMR-90 cells ± nutlin-3 (10 μM, 48 h). (E) Timing of cell death onset (D_O) ± 95% confidence interval in IMR-90 cells treated with erastin2 following pretreatment ± nutlin-3 (10 μM, 48 h). For nutlin-3-pretreated cells, the upper 95% confidence interval was not accurately resolved (indicated by the dotted line). (F) Cell death over time in HT-1080^N cells pretreated ± nutlin-3 (48 h) then treated with erastin2 ± mitoTEMPO. Unless otherwise indicated, data represent mean ± SD from three biological replicates. The brightness of the blots in A and D was increased uniformly to enable better



Supplemental Figure 3. p53-dependent transcription is necessary to suppress ferroptosis, Related to Figure 2. (A) Timing of cell death onset in HT-1080^{N} cells pretreated with nutlin-3 (10 μM) for various times. Data represent mean ± 95% confidence interval from two biological replicates. (B) p53 protein expression detected by immunohistochemistry following infection for 48 h with either an empty adenovirus (Ad-Empty) or an adenovirus directing the expression of Cre recombinase (Ad-Cre). Scale bar = 50 μm. (C) Relative gene expression following infection for 48 h with either Ad-Empty or Ad-Cre. (D) p53^{25,26} protein expression detected by immunohistochemistry following infection for 48 h with either Ad-Empty or Ad-Cre. Scale bar = 50 μm. (E) Relative gene expression following infection for 48 h with either Ad-Empty or Ad-Cre. (F) p53 and p21 levels in Caki-1 cells infected with a non-targeting shRNA (sh-NT) or shRNA targeting CDKN1A (sh-CDKN1A) ± nutlin-3 (10 μM). (G) SYTOX Green positive (SG^{+}) dead cell counts in Caki-1 cells infected and treated ± nutlin-3 for 48 h, as described in F, then treated ± erastin2 (1 μM) for 24 h. (H) p21 levels in HT-1080 cells infected with CMV-empty (Empty) or CMV-CDKN1A lentivirus for 48 h. (I) Representative images of HT-1080 cells infected as described in H and treated ± erastin2 (1 μM). Scale bar = 25 μm. Data in C, E and G represent mean ± SD from three biological replicates.



Supplemental Figure 4. p53 and GSH metabolism, Related to Figure 4. (A) Total glutathione levels in HT-1080 control and CDKN1A$^{KO1/2}$ cell lines measured using Ellman's reagent following pretreatment ± nutlin-3 (10 µM, 48 h) and treatment ± erastin2 (1 µM, 8 h). BD: below the limit of detection. (B) Unlabelled serine M+0 and labelled serine M+3 in HT-1080 control and TP53^{KO} cells pretreated ± nutlin-3 (10 µM, 48 h) then treatment ± erastin2 (1 µM, 8 h) in the presence of U-^{13}C-serine. Data in A and B represent mean ± SD from three independent biological replicates. (C) Model of p53 and p21 effects on the timing of lipid ROS accumulation and ferroptosis onset in response to system x_c^- inhibition and/or cystine deprivation.

Supplemental Experimental Procedures

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Scott Dixon (sjdixon@stanford.edu).

Experimental Model and Subject Details

Cell Lines and Culture Conditions

HT-1080 (CCL-121), U-2 OS (HTB-96), A549 (CCL-185), CAKI-1 (HTB-46), ACHN (CRL-1611), T98G (CRL-1697), NCI-H1299 (CRL-5803), SJSA-1 (CRL-2098), IMR-90 (CCL-186) and 293T (CRL-3216) were obtained from ATCC (Manassas, VA, USA). HT-1080 cells were cultured in DMEM Hi-glucose medium (Cat# MT-10-013-CV, Corning Life Science) supplemented with 1% non-essential amino acids (NEAAs) (Cat# 11140-050, Life Technologies). U-2 OS, CAKI-1 and IMR-90 cells were cultured in McCoy's 5A media (Cat# MT-10-050-CV, Corning Life Science). A549, ACHN, T98G and 293T cells were cultured in DMEM Hi-glucose media. NCI-H1299 (referred to as H1299) and SJSA-1 cells were cultured in RPMI 1640 media with L-glutamine (Cat# SH30027FS, GE Healthcare). Polyclonal Nuc::mKate2-expressing HT-1080 cells (denoted HT-1080^{N}), as well as similar U-2 OS^{N}, A549^{N} and T98G^{N} cell lines were described previously (Forcina et al., 2017).

Method Details

Chemicals and Reagents:

Erastin2 (compound 35MEW28 reported in (Dixon et al., 2014)) was synthesized by Acme Bioscience (Palo Alto, CA, USA). Bortezomib (Cat# NC0587961), buthionine sulfoximine (Cat# AC23552-0010) and C11 BODIPY 581/591 (Cat# D3861) were from Thermo Fisher Scientific (hereafter Thermo Fisher). Nutlin-3 (Cat# S1061) was from Selleck Chemicals (Houston, TX, USA). Ferrostatin-1 (Cat# SML0583), mitoTEMPO (Cat# SML0737) and *tert*-butylhydroquinone (tBHQ, Cat# 112941) were from Sigma-Aldrich. Palbociclib was from SelleckChem (Cat# S1116, the kind gift of Dr. Julien Sage). Buthionine sulfoximine was dissolved directly into cell media. C11 BODIPY 581/591 was prepared as a stock solution in methanol and stored prior to use at -20˚C. All other compounds were prepared as stock solutions in DMSO and stored prior to use at -20˚C.

CRISPR/Cas9 gene editing

HT-1080 *TP53*^{KO} and *CDKN1A*^{KO} cell lines were generated as follows. A genomic region corresponding to exon 3 of human *TP53* and exon 2 of human *CDKN1A* was input into the CRISPR Design Tool (MIT, crispr.mit.edu). *TP53*: The following guides were selected based on a high quality score (97/100): Fwd- 5'-CCATTGTTCAATATCGTCCG, Rev- 3'-CGGACGATATTGAACAATGG. Overhangs (underlined) were added to each guide, yielding the following sequences: Fwd- 5'-CACCGCCATTGTTCAATATCGTCCG, Rev- 3'-AAACCGGACGATATTGAACAATGGC**.** *CDKN1A***:** The following guides were selected based on a high quality score (86/100): Fwd- 5'- TACCCTTGTGCCTCGCTCAG, Rev- 3'-CTGAGCGAGGCACAAGGGTA. Overhangs (underlined) were added to each guide, yielding the following sequences: Fwd- 5'- CACCGTACCCTTGTGCCTCGCTCAG, Rev- 3'- AAACCTGAGCGAGGCACAAGGGTAC**.** Guide RNAs were phosphorylated and annealed using T4 polynucleotide kinase (Cat# M0201S, New England Biolabs). The annealing reaction was incubated for 30 min at 37ºC, 95ºC for 5 min, then ramped down to 25ºC at a rate of 5ºC/min. Annealed primers were then diluted 1:200 in nuclease free water. Diluted annealed primers were then cloned into the pSpCas9(BB)-2A-GFP (PX458**,** Addgene, a kind gift from Dr. Jan Carette) plasmid using T4 DNA ligase (Cat# EL0014, Thermo Fisher). The ligation reaction was incubated for 6 cycles of 37ºC for 5 min followed by 21ºC for 5 min. To remove linearized DNA, the ligation product was treated with Plasmid-Safe DNase (Cat# E3101K, Epicenter) and incubated at 37ºC for 30 min, then 70ºC for 30 min. DH5α *E. coli* were transformed with the resulting ligation product and grown under ampicillin selection. Plasmid DNA was purified using the QIAprep Spin Miniprep Kit (Cat# 27106, Qiagen). Next, 1 µL of PolyJet (Cat# SL100688, SignaGen Laboratories) transfection reagent was used to transduce 50,000 HT-1080 cells seeded in 6-well format with 1 µg of pSpCas9(BB)-2A-GFP-sgRNA plasmids. Cells were incubated for 24 h, then GFP-expressing cells were sorted into single wells of a 96-well dish containing DMEM media with 30% fetal bovine serum on a BD InFlux cell sorter (Stanford Shared FACS Facility). Plates were monitored for three weeks for colony outgrowth. Colonies were dissociated using 0.25% trypsin-EDTA (Cat# 25200114, Thermo Fisher Scientific) and expanded up to 24-well plates, then to T-75 flasks. Genomic DNA (gDNA) was harvested from 0.5-2 x 10^6 cells using the QIAamp DNA Mini Kit (Cat# 51304, Qiagen). For validation of *TP53*^{KO} clones, a ~300 bp fragment from the *TP53* locus was amplified by PCR using the following primers: 5'-GCCAGGCATTGAAGTCTCAT-3' and 5'-GTCCCAAGCAATGGATGATT-3'. The PCR product was purified using the QIAquick PCR Purification Kit (Cat# 28106, Qiagen) and sequenced to screen for genetic disruption of the *TP53* locus with the following primer: 5'-TTCTGGGAAGGGACAGAAGATGACA-3'. A colony was identified in which a single base was

inserted at the expected cut site that resulted in a downstream premature stop codon. This clone was expanded and the absence of p53 protein was confirmed by Western blotting. For validation of *CDKN1A*^{KO} clones, cells growing in a T75 flask were dissociated using 0.25% trypsin-EDTA and approximately 1x10^6 cells were collected. Cells were pelleted and lysed in 9 M urea. Lysates were prepared and immunoblotted as described below (see *Immunoblotting)* to identify knockout cell lines.

Cell viability experimental design and set-up

Most experiments testing the effect of p53 stabilization on cell death used a pretreatment phase (24 or 48 h), with cells treated with either vehicle control (DMSO) or MDM2 inhibitor (e.g. nutlin-3). In all experiments a nutlin-3 concentration of 10 µM was used. Cell death was not assessed during this pretreatment phase. The unobserved pretreatment phase was followed by a treatment phase of 48 h where cell death observations were made, most often using scalable time-lapse analysis of cell death kinetics (STACK, (Forcina et al., 2017)) and cell lines expressing nuclear-localized mKate2 (e.g. control, *TP53*^{KO} and *CDKN1A*$^{KO1/2}$ HT-1080^N cells, Caki-1^N, H1299^N, etc). In some experiments, unmodified cells were employed and cell death was examined by SYTOX Green uptake alone (SJSA-1, IMR-90, mouse cancer cell lines). The detailed experimental set-up was as follows. On day 1, cells were seeded into clear bottom black 96-well plates (Cat# 07-200-588, Fisher Scientific). Cell seeding was optimized to ensure approximately equal cell numbers at the end the pretreatment phase (e.g. accounting for the fact that nutlin-3 treatment would arrest the proliferation of p53 wild-type cell lines but not p53 mutant cell lines). The following number of cells/well were used, for cells destined to be pretreated with either DMSO (D) or nutlin-3 (N): control HT-1080^N (D: 2000, N:4000), *TP53*^{KO} HT-1080^N (D: 1500, N:1500), *CDKN1A*$^{KO1/2}$ HT-1080^N (D: 2000, N:3000), U-2 OS^N (D: 2000, N: 4000), ACHN^N (D: 3000, N: 5000), Caki-1^N (D: 2000, N: 4000), H1299^N (D: 4000, N: 4000), T98G^N (D: 2500, N: 2500), A549^N (D: 5000, N: 5000), SJSA-1 (D:2000, N:4000), IMR-90 (D:5000, N:5000), *Trp53*$^{+/+}$ MEFs (D: 4000, N: 4000) and *trp53*$^{-/-}$ MEFs (D: 2000, N: 2000). On day 2, the start of the pretreatment phase, medium was exchanged and replaced with that containing either DMSO or MDM2 inhibitor (i.e. typically nutlin-3, 10 µM). On day 4 the medium was changed again and cells from both pretreatment conditions were then incubated in medium containing lethal compounds ± cell death inhibitors (e.g. ferrostatin-1) for an additional 48 h, until day 6. Note that either DMSO or nutlin-3 from the pretreatment phase was maintained in the medium during the treatment (observed) phase. Pretreatments were also performed using palbociclib (2 µM, 48 h). Cells for DMSO pretreatment were seeded at a density of 2000 cells/well, while those for palbociclib pretreatment were seeded at 3000 cells/well.

Cell death assessment using STACK

Cell death analysis was typically performed using STACK as follows. Following compound pretreatment or infection (e.g. adenovirus, shRNA), lethal compounds were added together with SYTOX Green viability dye (Cat# S7020, Life Technologies) at a final concentration of 22 nM. Cells were imaged at 2 or 4 h intervals for 48 h using the IncuCyte live cell analysis system (Essen BioScience). For cell lines stably expressing Nuc::mKate2 (e.g. HT-1080^N, ACHN^N), both mKate2 positive (mKate2$^+$) and SYTOX Green positive (SG$^+$) objects were counted using the IncuCyte ZOOM Live-Cell Analysis System software. Lethal fraction scores were computed for each time point, as described (Forcina et al., 2017). Lag exponential death (LED) curve fits to lethal fraction scores over time were obtained using Prism 6.0h (GraphPad, La Jolla, CA, USA), as described (Forcina et al., 2017). Time of cell death onset (a parameter value referred to as D_O) was obtained from LED curves fits, as described (Forcina et al., 2017). For experiments using cells not expressing Nuc::mKate2 cell death (SJSA-1, IMR-90, MEFs), cell death was measured by counting SG$^+$ objects over time. In some experiments, SG$^+$ counts were normalized between samples on a common scale using differences in starting cell confluence, inferred from phase-contrast images acquired in parallel, as a metric. Since long-dead cells can release SG (Forcina et al., 2017), the maximum SG$^+$ counts from the start of each time course were used in these calculations.

Transient cystine deprivation

The day before experiment, HT-1080^N control (60,000 cells/well for DMSO pretreatment and 120,000 cells/well for nutlin-3 pretreatment) and *TP53*^{KO} (40,000 cells/well for both pretreatments; we note that *TP53*^{KO} populations expand more quickly than control populations) cells were seeded in 6-well plates. The next day, cells were pretreated in cystine-replete medium with DMSO or nutlin-3 (10 µM). After two days, the cells were washed 3 times with HBSS, then treated with cystine-free medium + 20 nM SYTOX Green (except for the 0 h treatment, which was treated with cystine-replete medium + 20 nM SYTOX Green) + DMSO or nutlin-3 (10 µM). At a desired time point of cystine repletion, plates were imaged (IncuCyte Zoom) prior to replacing cystine-free medium + 20 nM SYTOX Green + DMSO or nutlin-3 (10 µM) with cystine-replete medium + 20 nM SYTOX Green. Note: population cell death was computed using the lethal fraction scoring approach which controls for the loss of SYTOX Green signal from long dead cells (Forcina et al., 2017). Thus, a decrease in the lethal fraction score over time from some maximum value can only be explained by an increase in the live cell numbers within the population (Forcina et al., 2017). Images were analyzed as described above.

PrestoBlue

In one experiment cell viability was assayed using PrestoBlue (Cat# A13262, Life Technologies). Cells were pre-treated with DMSO or nutlin-3 (10 μM) for 48 h as described above. Lethal compounds were then added and cells were incubated for a further 16 h. 10% final (v/v) PrestoBlue reagent was then added to existing media and mixed five times. Following 30 min incubation in a tissue culture incubator at 37°C, PrestoBlue signal was measured using a Synergy Neo2 reader (BioTek Instruments, Winooski, VT) at ex/em 530/590 nm. Background fluorescence from medium-only controls + 1x PrestoBlue was subtracted from all values and samples were normalized to an internal control treated with DMSO.

Adenovirus infection

For Western blot and RT-qPCR analysis, on day 1 murine $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ and $Kras^{LA2/+};Trp53^{LSL\text{-}25,26/LSL\text{-}25,26}$ lung tumor cell lines cells were seeded into 6-well dishes. Given that we expected cells re-expressing p53 to stop proliferating, we initially seeded different numbers of cells such that by the end of 48 h we had roughly equivalent numbers for downstream assays. Thus, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Empty were seeded at 1 x 10^5 cells/well, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Cre were seeded at 2 x 10^5 cells/well, and $Kras^{LA2/+};Trp53^{LSL\text{-}25,26/LSL\text{-}25,26}$ cells destined for infection with either Ad-Empty or Ad-Cre were seeded at 1 x 10^5 cells/well. On day 2, cells were infected with either Ad5CMVempty (Ad-Empty, Cat# VVC-U of Iowa-272) or Ad5CMVCre (Ad-Cre, Cat# VVC-U of Iowa-5), obtained from the University of Iowa Viral Vector Core, at an M.O.I. of 100.

Cell death assays were conducted in 96-well plates. On day 1, cells were seeded at different numbers for reasons described above. Thus, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Empty were seeded at 5000 cells/well, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Cre were seeded at 7500 cells/well, and $Kras^{LA2/+};Trp53^{LSL\text{-}25,26/LSL\text{-}25,26}$ cells destined for infection with either Ad-Empty or Ad-Cre were seeded at 4000 cells/well. On day 2 cells were infected using virus at an M.O.I. of 100. On day 4, the medium was replaced with cell death assay medium containing SYTOX Green and test compounds as described above.

Immunofluorescence

On day 1, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ and $Kras^{LA2/+};Trp53^{LSL\text{-}25,26/LSL\text{-}25,26}$ cells were seeded on glass cover slips. $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Empty were seeded at 1 x 10^5 cells/well, $Kras^{LA2/+};Trp53^{LSL\text{-}wt/LSL\text{-}wt}$ cells destined for infection with Ad-Cre were seeded at 2 x 10^5 cells/well, and $Kras^{LA2/+};Trp53^{LSL\text{-}25,26/LSL\text{-}25,26}$ cells destined for infection with either Ad-Empty or Ad-Cre were seeded at 1 x 10^5 cells/well. On day 2, cells were infected as described above and incubated at 37°C for a further 48 h. On day 4, cells were washed with PBS and fixed in 4% paraformaldehyde for 15 min at room temperature. Cover slips were then washed three times with PBS and stored in PBS at 4°C. Cells were then permeabilized with 0.25% Triton-X in PBS for 10 min at room temperature and washed twice in PBS. CM5 anti-p53 antibody was diluted 1:200 in PBS with 1% BSA and added to cover slips for 30 min at 37˚C. Cells were then washed three times in PBS and a secondary antibody mix prepared by diluting donkey-anti-rabbit 488 antibody (1:2000) (Cat# A21206, Life Technologies) and DAPI (1:10,000) (Cat# D1306, Life Technologies) in PBS was added for 45 min. Slides were then washed three times with PBS and a drop of ProLong Gold antifade reagent (Cat# P36930, Thermo Fisher) was added and the coverslips were mounted on slides. Slides were allowed to dry overnight and sealed with nail polish. Images were acquired using a Zeiss Observer Z1 confocal microscope. Images were processed in ImageJ (National Institutes of Health, Bethesda, MA).

Image Analysis

Images were processed in Image J (version 1.50i) or Adobe Photoshop (Adobe Systems, San Jose, CA).

Immunoblotting

Cells were washed twice in HBSS (Cat# 14025-134, Life Technologies) and harvested using a cell scraper. Cell pellets were lysed in 9M urea and sonicated ten times using one-second pulses at maximum amplitude on a Fisher Scientific Model 120 Sonic Dismembrator (Thermo Fisher). Lysates were centrifuged at 15,000 rpm at room temperature for 15 min and supernatants were removed to exclude debris. Lysates were quantified by Bradford Assay using the Bio-Rad Protein Assay reagent (Cat# 5000002, Bio-Rad). Samples were prepared using NuPage Reducing Agent (Cat# NP0009, Thermo Fisher), NuPage LDS Sample Buffer (Cat# NP0007, Thermo Fisher) and dithiothreitol. Samples were incubated at 70˚C and run on pre-cast NuPage SDS 4-12% gradient gels in NuPage MES Running Buffer (Cat# NP0002, Thermo Fisher).

For xCT immunoblotting, cells were washed twice in HBSS (Cat# 14025-134, Life Technologies) and harvested with a cell scraper. Cell pellets were lysed in RIPA buffer containing 0.1% SDS (10mM Tris pH 7.5, 150mM NaCl, 1mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 1% Triton X100) for 1 h on ice. Lysates were then sonicated as described above. Lysates were centrifuged for 20 min at 15,000 rpm at 4ºC. Supernatants were collected and quantified using a Pierce BCA Protein Assay kit (Cat# 23225, Thermo Fisher). Samples were prepared using 4x Laemmli buffer (Cat# 1610747, Bio-

Rad) and incubated 10 min at room temperature. Samples were then run on pre-cast 4-15% polyacrylamide gels (Cat# 4561084, Bio-Rad).

Gels were transferred using an iBlot Dry Blotting System (Cat# IB21001, Thermo Fisher). Membranes were blocked in Odyssey PBS Blocking Buffer (Cat# 927-40010, Li-Cor) for one hour and probed with primary antibodies. Primary antibodies used were against actin (Cat# SC-1616, I-19, Santa Cruz), human p53 (Cat #SC-126, DO-1, Santa Cruz), murine p53 (Cat# SC-6243, FL-393, Santa Cruz), murine p53 (Cat#NCL-L-p53-CM5p, CM5, Leica), xCT (Cat# 12691, D2M7A, Cell Signaling Technology), and p21 (Cat# 2947, 12D1, Cell Signaling Technology). Membranes were probed overnight at 4˚C or for 1 hour at room temperature with rocking. Membranes were washed three times for 5 min in Tris buffered saline (Cat# 0788, ISC BioExpress) with 0.1% Tween 20 (TBST) at room temperature with rocking. Samples were probed with secondary antibodies (Donkey anti-goat-680 Cat# 926-68024, Donkey anti-goat-800 Cat# 926-32214, Donkey anti-rabbit-680 Cat# 926-68023, Donkey anti-rabbit-800 Cat# 926-32213, Donkey anti-mouse-680 Cat# 926-68022, Donkey anti-mouse-800 Cat# 926-32212, Li-Cor) in Odyssey Buffer (Li-COR, Cat# 927-40100) with 0.1% SDS and 0.4% Tween 20 for 1 h at room temperature with rocking. Membranes were washed three times for 5 min in TBST Membranes were then imaged using a Li-COR Odyssey CLx imager.

Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

Following treatment, cells were washed twice in PBS, and scraped to harvest. Cell lysates were harvested and RNA extracted using a Qiashredder extraction column (Qiagen, Cat# 79654) and the RNeasy Plus RNA Extraction Kit (Cat# 74134, Qiagen). cDNA was generated using the TaqMan Reverse Transcriptase Kit according to the manufacturer's instructions (Cat# N8080234, TaqMan). Quantitative PCR reactions were prepared with SYBR Green Master Mix (Cat# 4367659, Life Technologies) and run on an Applied Biosystems QuantStudio 3 real-time PCR machine (Thermo Fisher). Relative transcript levels were calculated using the ΔΔCT method and normalized to the *ACTB* gene. qPCR primer sequences are provided in the Supplemental Materials.

siRNA Gene Knockdown

HT-1080 cells were reverse-transfected using an siRNA targeting human *SLC7A11* (Hs_SLC7A11_2 FlexiTube siRNA, Cat# SI00104902, Qiagen). AllStars Negative Control siRNA (Cat# 1027280, Qiagen) was used as a negative control. AllStars Hs Cell Death siRNA (Cat# 1027298, Qiagen) was used as a positive control for transfection efficiency. Transfection mixes were prepared using 5 nM siRNA and 2 µL Lipofectamine RNAiMAX Transfection Reagent (Cat# 13778075, Life Technologies) to total of 200 µL in Opti-MEM Reduced Serum Media (Cat# 31985-062, Life Technologies). Diluted siRNA was added to diluted lipofectamine. The combined mixture was added directly to empty 6 well dishes and incubated for 15 min at room temperature. 1 x 10^5 HT-1080 cells in suspension were then plated directly onto the transfection mixture and swirled to combine. Cells were incubated for 48 h prior to assays and plates were only used in assays if all cells in the Hs Cell Death siRNA positive control wells were dead.

shRNA and cDNA Overexpression Constructs

The PLKO.1 shRNA lentiviral plasmid targeting *CDKN1A* was from Sigma-Aldrich (Cat# TRCN0000287091). The PLKO.1 non-targeting shRNA control plasmid was from Addgene (Cat# 1864). cDNA from human *CDKN1A* (Genbank Accession # CV025498.1) in a pDONR223 vector was obtained from the human ORFeome V7.1 library (clone ID 2821049, a kind gift of Dr. Aaron Gitler). The cDNA was cloned into the pLenti-CMV-Puro DEST vector (Cat# w118-1, Addgene) using Gateway LR Clonase II Enzyme Mix (Cat# 11791-020 Thermo Fisher) and confirmed by sequencing. Plasmid DNA was isolated using the QIAprep Spin Miniprep Kit (Cat# 27106, Qiagen).

Lentiviral Production

Lentiviruses were generated in 293T cells as follows. 0.5 x 10^6 293T cells were seeded in a 6-well dish the day before the transfection. The following day, cells were transfected with 1000 ng of plasmid DNA, along with 250 ng pMD2.G (Cat# 12259, Addgene) and 750 ng psPax2 (Cat# 12260, Addgene) lentiviral packaging plasmids. 3 µL PolyJet (Cat# SL100688, SignaGen Laboratories) transfection reagent diluted in plain DMEM was added to diluted plasmid DNA to a final volume of 100 µL and incubated for 15 min at room temperature. The transfection mixture was then added dropwise to cells. After 24 h, lentivirus-containing media was harvested by collecting media from cells three times at > 8 h intervals. Viral media was then filtered through a 0.45 µM PVDF (Cat# SLHV033RS, Millex) syringe-driven filter unit and frozen at -80ºC until use.

CDKN1A shRNA silencing experiment

Caki-1 cells were seeded in a 96-well dish at 4000 (cells treated with control shRNA constructs and nutlin-3) or 2000 (all other treatments) cells/well and infected with lentiviruses carrying non-targeting shRNAs (sh-NT) or shRNAs targeting *CDKN1A* at an M.O.I. of ~1 in media containing 8 µg/mL Polybrene (source). Infected cells were then spun at 1000

rpm for 1 h at room temperature. The next day, virus-containing medium was removed and cells were incubated in medium containing puromycin (10 µg/mL) and DMSO or nutlin-3 (10 µM) for a further 24 h. Media was then removed and replaced with media containing SYTOX Green (0.022 µM), DMSO or nutlin-3 (10 µM), and DMSO or erastin2 (1 µM), and cell death was assayed for the subsequent 48 h using time-lapse imaging. Cell death was assessed by counting SYTOX Green positive cells over time.

CDKN1A overexpression experiment

HT-1080 cells were seeded in a 96 well dish at a density of 2000 cells/well. The following day, cells were infected with lentiviruses carrying an empty vector (pLenti-CMV-Puro-DEST) or a lentivirus directing expression of *CDKN1A* cDNA under a CMV promoter (pLenti-CMV-Puro-*CDKN1A*), generated as described above. Cells were infected at an M.O.I. of ~1 in media containing 8 µg/mL polybrene and spun at 1000 rpm for 1 h at room temperature. The next day, virus-containing medium was removed and replaced with medium containing 10 µg/mL puromycin. After 24 h, cells were treated with SYTOX Green (0.022 µM) and DMSO or erastin2 (1 µM) and cell death was assayed using time-lapse imaging of SYTOX Green positive cells.

To collect cell material for p21 immunoblotting, 1 x 10^5 HT-1080 cells/well were seeded in a 6 well dish. The following day, cells were infected with lentiviruses, as described above, in media containing 8 µg/mL polybrene. The following day, virus-containing medium was removed and replaced with medium containing 10 µg/mL puromycin. After 24 h, media was removed, cells were washed twice in PBS, and cells were scraped, spun at 1500 rpm for 2 min, and cell pellets harvested for Western blotting.

Cell Cycle Analysis

The day before the start of the experiment, 1 x 10^5 (DMSO) or 2 x 10^5 (nutlin-3, palbociclib) HT-1080 cells/well were seeded into 6-well plates. The next day, cells were treated with DMSO, nutlin-3 (10 µM, 48 h) or palbociblib (2 µM, 24 h). At the end of the treatment phase, cells were dissociated with 0.25% trypsin-EDTA (Cat# 25200114, Thermo Fisher), centrifuged at 1000 x g for 5 min, washed twice in HBSS (Cat# 14025-134, Life Technologies), and fixed in 70% ethanol. Cells were stored at 4ºC for up to three weeks. Cells were then pelleted, washed in DPBS lacking calcium or magnesium (Cat# CV-21-031, Corning), and treated with RNAse A (Cat# 19101, Qiagen) at 1:100 (v/v) and propidium iodide (Cat# P3566, Thermo Fisher) at 1:20 (v/v). Cells were then incubated 30 min at 37ºC, centrifuged, washed twice in DPBS, resuspended in DPBS, and strained through a filter-top tube (Cat# 352235, Corning). Cell cycle status was quantified using a BD FACSCalibur flow cytometer. Data were processed using FlowJo v10.1 (FlowJo, LLC).

Glutamate Release Assay

Glutamate release was assayed as described (Dixon et al., 2014). The day before the experiment, 1-2 x10^5 adherent cells were seeded in a 6-well dish. The next day, cells were washed twice in cystine uptake buffer (137 mM choline chloride, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$,5 mM D-glucose, 0.7 mM K_2HPO_4, 10 mM HEPES, 300 µM cystine, pH 7.4). Uptake buffer containing DMSO or nutlin-3 (10 µM) and DMSO or erastin2 (1 µM) was then added to cells and incubated for 60 min at 37ºC. Cell medium was then collected and added to a 96-well assay plate. For normalization purposes, cells were trypsinized in 0.25% trypsin-EDTA (Cat# 25200114, Thermo Fisher) and cell number was quantified using a Cellometer Auto T4 Bright Field Cell Counter (Nexcelcom, Lawrence, MA). Glutamate release was detected using the Amplex Red Glutamic Acid/Glutamate Oxidase Assay kit (Cat# A-12221, Thermo Fisher) as per the manufacturer's instructions. 10 µM H_2O_2 and 25 µM L-glutamate were included as positive controls. Fluorescence readings were recorded at ex/em 530/590 on a Synergy Neo2 reader (BioTek). Background fluorescence from blank uptake medium was subtracted and samples were normalized to cell number.

C11 BODIPY 581/591 Imaging

HT-1080 cells were seeded on glass coverslips placed in 6 well dishes at a density of 1x10^5 (DMSO treated) or 2x10^5 cells/well (nutlin-3 pretreated). Cells were pretreated with DMSO or nutlin-3 (10 µM) for 48 h. Media was then removed and replaced with media containing DMSO or nutlin-3 (10 µM), consistent with the respective pretreatment condition, and either DMSO or erastin2 (1 µM) for 10 h. At this point, cell media was aspirated and cells were washed with HBSS. A 5 µM working solution of C11-BODIPY was prepared in HBSS and added to cells for 10 min at 37ºC. The C11-BODIPY mixture was then aspirated and 1 mL of fresh HBSS was added to cells. To prepare cells for imaging, 25 µL of HBSS was pipetted onto a microscope slide lined with parafilm. Coverslips holding stained cells were then lifted out of plates using a needle tip and inverted onto prepared slides. Slides were then sealed with melted Vaseline. Slides were imaged on a Zeiss Observer Z1 confocal microscope and images were processed in ImageJ. Brightness for all images was auto-adjusted based on images with the brightest signal.

Biochemical Glutathione Detection Assay

Total glutathione was assayed as described (Dixon et al., 2014). Following treatment in 6-well dishes, cells were washed once with HBSS (Cat# 14025-134, Life Technologies) and collected into MES buffer with 1 mM EDTA using a cell scraper. Samples were sonicated ten times with one second pulses at maximum amplitude on a Fisher Scientific Model 120 Sonic Dismembrator (Thermo Fisher). Lysates were centrifuged at 14,000 rpm for 15 min at 4ºC. Supernatants were collected and protein was quantified by Bradford assay using the Bio-Rad Protein Assay reagent (Cat# 5000002, Bio-Rad). To deproteinate lysates, an equal volume of 12.5M metaphosphoric acid was added and incubated for 5 min at room temperature. Lysates were then centrifuged at 15,000 rpm for 3 min at room temperature and the resultant supernatants collected and stored at -20ºC. Total glutathione was measured using the Cayman Glutathione Assay kit (Cat# 703002, Cayman Chemical) per the manufacturer's instructions. Assay reading was performed on a Synergy Neo2 reader (BioTek). Results were normalized to total protein concentration for each sample.

Metabolic Flux Analysis

HT-1080 control (75,000 c/w for DMSO, 150,000 c/w for nutlin-3), *TP53*^{KO} (50,000 c/w for both DMSO and nutlin-3) and *CDKN1A*^{KO1} cells (75,000 c/w for DMSO, 100,000 c/w for nutlin-3) were seeded overnight in 6-well dishes in RPMI 1640 medium (Cat# SH30027FS, Fisher Scientific) containing 10% FBS and 1x Pen/Strep. The next day, pretreatment was initiated with DMSO or nutlin-3 (10 μM) for 48 h in RPMI 1640 medium. After 48 h, media was removed and cells were then incubated for 8 h in RPMI medium lacking glucose, serine, and glycine (Cat# R9660-02, TEKnova) supplemented with 30 mg/L L-Serine-^{13}C3 (Cat# 604887**,** Sigma-Aldrich) and 2000 mg/L D-glucose (Cat# G54000, Sigma-Aldrich) ± erastin2 (1 μM). DMSO or nutlin-3 (10 μM) conditions were maintained from the pretreatment phase. Unlabeled controls were incubated for 8 h in standard RPMI 1640 medium with DMSO or nutlin-3 (10 μM). Following the 8 h treatment phase, cells were washed twice in cold PBS and fixed in 80% cold LC/MS grade acetonitrile (Cat #A955-500, Fisher Scientific) for 5 min on ice. Cells were then scraped to collect, sonicated three times for 15 sec in a water bath, spun at 12,000 rpm for 10 min, and the resulting supernatants were stored at -80ºC. The resulting pellet was resuspended in 100 μl of 0.2 M NaOH and heated for 20 min at 95^{o}C with vortexing every 5 min. The dissolved proteinaceous solution was pelleted at maximum speed for 5 min and protein concentration in the supernatant was determined by BCA assay.

Quantitative LC-ESI-MS/MS analysis of cell extracts was performed using an Agilent 1290 UHPLC system equipped with an Agilent 6545 Q-TOF mass spectrometer (Santa Clara, CA, US). A hydrophilic interaction chromatography method (HILIC) with an BEH amide column (100 x 2.1 mm i.d., 1.7 μm; Waters) was used for compound separation at 35 °C with a flow rate of 0.3ml/min. The mobile phase A consisted of 25 mM ammonium acetate and 25mM ammonium hydroxide in water and mobile phase B was acetonitrile. The gradient elution was 0–1 min, 85 % B; 1–12 min, 85 % B → 65 % B; 12–12.2 min, 65 % B-40%B; 12.2-15 min, 40%B. After the gradient, the column was re-equilibrated at 85%B for 5min. The overall runtime was 20 min and the injection volume was 5 μL. Agilent Q-TOF was operated in negative mode and the relevant parameters were as listed: ion spray voltage, 3500 V; nozzle voltage, 1000 V; fragmentor voltage, 125 V; drying gas flow, 11 L/min; capillary temperature, 325 °C, drying gas temperature, 350 °C; and nebulizer pressure, 40 psi. A full scan range was set at 50 to 1600 (m/z). The reference mass were 119.0363 and 980.0164. The acquisition rate was 2 spectra/s. Isotopologues extraction was performed in Agilent Profinder B.08.00 (Agilent technologies). Retention time (RT) of each metabolite was determined by authentic standards (Table). The mass tolerance was set to +/-15 ppm and RT tolerance was +/-0.2 min.

Name	Formula	Mass(Da)	RT(Min)	METLIN	HMP	KEGG
Glycine	$C_2H_5NO_2$	75.032	6.96	20	HMDB00123	C00037
L-Serine	$C_3H_7NO_3$	105.043	7.56	30	HMDB00187	C00065
Glutathione	$C_{10}H_{17}N_3O_6S$	307.084	8.78	44	HMDB00125	C00051
Glutathione, oxidized	$C_{20}H_{32}N_6O_{12}S_2$	612.152	13.42	45	HMDB03337	C00127

Graphing and Figure Assembly

Graphing and all other statistical analyses were performed using GraphPad Prism 6.0h. The Results and individual Figure Legends contain additional statistical details. Figures were assembled using Adobe Illustrator.

Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-p53 (mouse) (CM5)	Leica	Cat# P53-CM5P-L

Donkey-anti-rabbit 488	Life Technologies	Cat# A21206
DAPI	Life Technologies	Cat# D1306
Anti-Actin (I-19)	Santa Cruz	Cat# SC-1616
Anti-p53 (human) (DO-1)	Santa Cruz	Cat# SC-126
Anti-p53 (mouse) (FL-393)	Santa Cruz	Cat# SC-6243
Anti-p53 (mouse) CM5	Leica	Cat# P53-CM5P-L
Anti-xCT (D2M7A)	Cell Signaling Technology	Cat# 12691
Anti-p21 (12D1)	Cell Signaling Technology	Cat# #2947
IRDye 680LT Donkey anti-Goat IgG	Licor	Cat# 926-68024
IRDye® 800CW Donkey anti-Rabbit IgG	Licor	Cat# 926-32213
IRDye® 800CW Donkey anti-Mouse IgG	Licor	Cat# 926-32212
IRDye® 680LT Donkey anti-Rabbit IgG	Licor	Cat# 926-68023
IRDye® 680LT Donkey anti-Mouse IgG	Licor	Cat# 926-68022
Bacterial and Virus Strains		
NucLight Red lentivirus reagent (EF1a, Puro) Nuclear-localized mKate2 (Nuc::mKate2)	Essen BioSciences	Cat# 4265
Ad5CMVempty	University of Iowa Viral Vector Core	Cat# VVC-U of Iowa-272
Ad5CMVCre	University of Iowa Viral Vector Core	Cat# VVC-U of Iowa-5
Biological Samples		
N/A		
Chemicals, Peptides, and Recombinant Proteins		
Sytox Green	Life Technologies	Cat# S7020
Erastin (Compound 13MEW76 in (Dixon et al., 2014))	(Dixon et al., 2014)	N/A
Erastin2 (Compound 35MEW28 in (Dixon et al., 2014))	(Dixon et al., 2014)	N/A
Buthionine sulfoximine	Fisher Scientific	Cat# AC23552-0010
Ferrostatin-1	Sigma-Aldrich	Cat# SML0583
mitoTEMPO	Sigma-Aldrich	Cat# SML0737
Nutlin-3	Selleck Chemicals	Cat# S8059
MI-773	Selleck Chemicals	Cat# S8059
Palbociclib	Selleck Chemicals	Cat# S1579
Etoposide	Thermo Fisher Scientific	Cat# 12-261-00
DAPI	Life Technologies	Cat# D1306
Polybrene	Sigma-Aldrich	Cat# H9268-5G
Puromycin	Life Technologies	Cat# A11138-03
PolyJet	SigmaGen Laboratories	Cat#SL100688
Propidium Iodide	Thermo Fisher	Cat# P3566
RNAse A	Qiagen	Cat#19101
Lipofectamine RNAiMAX Transfection Reagent	Life Technologies	Cat # 13778075
Critical Commercial Assays		
QIAshredder RNA Extraction Column Kit	Qiagen	Cat# 79654
RNeasy Plus RNA Extraction Kit	Qiagen	Cat# 74134
TaqMan Reverse Transcriptase Kit	TaqMan	Cat# N8080234
Cayman Glutathione Kit	Cayman Chemical	Cat # 703002
QIAprep Spin Miniprep Kit	Qiagen	Cat# 27106
QIAquick PCR Purification Kit	Qiagen	Cat# 28106

SYBR Green Master Mix	Life Technologies	Cat# 4367659
Gateway LR Clonase II Enzyme Mix	Thermo Fisher	Cat# 11791-020
Bradford Assay Kit	Bio-Rad	Cat# 5000002
BCA Protein Assay Kit	Thermo Fisher	Cat# 23225
Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit	Thermo Fisher	Cat# A-12221
PrestoBlue Cell Viability Reagent	Thermo Fisher	Cat# A13261
Deposited Data		
N/A		
Experimental Models: Cell Lines		
HT-1080	ATCC	CCL-121
HT-1080^{N}	(Forcina et al., 2017)	N/A
HT-1080 *Control*	This paper	N/A
HT-1080 *TP53*^{KO}	This paper	N/A
HT-1080 *CDKN1A*^{KO1}	This paper	N/A
HT-1080 *CDKN1A*^{KO2}	This paper	N/A
U-2 OS	ATCC	HTB-96
U-2 OS^{N}	(Forcina et al., 2017)	N/A
T98G^{N}	(Forcina et al., 2017)	N/A
A549^{N}	(Forcina et al., 2017)	N/A
Caki-1	ATCC	HTB-46
Caki-1^{N}	This paper	N/A
ACHN	ATCC	CRL-1611
ACHN^{N}	This paper	N/A
NCI-H1299 (referred to as H1299 in the manuscript)	ATCC	CRL-5803
NCI-H1299^{N}	This paper	N/A
Kras$^{LA2/+}$*; Trp53*$^{LSL\text{-}wt/LSL\text{-}wt}$	This paper	N/A
Kras$^{LA2/+}$*; Trp53*$^{LSL\text{-}25,26/LSL\text{-}25,26}$	This paper	N/A
293T	ATCC	CRL-3216
SJSA-1	ATCC	CRL-2098
IMR-90	ATCC	CRL-186
Trp53$^{+/+}$ primary MEFs	(Johnson et al., 2005)	N/A
Trp53$^{-/-}$ primary MEFs	(Johnson et al., 2005)	N/A
Experimental Models: Organisms/Strains		
N/A		
Oligonucleotides		
Human *ACTB* qPCR forward primer [ATCCGCCGCCCGTCCACA]	(Van Nostrand et al., 2014)	N/A
Human *ACTB* qPCR reverse primer [ACCATCACGCCCTGGTGCCT]	(Van Nostrand et al., 2014)	N/A
Human *CDKN1A* qPCR forward primer [CACCGAGACACCACTGGAGG]	(Huang et al., 2015)	N/A
Human *CDKN1A* qPCR forward primer [GAGAAGATCAGCCGGCGTTT]	(Huang et al., 2015)	N/A
Human *MDM2* qPCR forward primer [GAATCATCGGACTCAGGTACATC]	Primerbank	Accession NM_002392.5
Human *MDM2* qPCR reverse primer [TCTGTCTCACTAATTGCTCTCCT	Primerbank	Accession NM_002392.5
Human *SLC7A11* qPCR forward primer [GGGCATGTCTCTGACCATCT]	(Martin and Gardner, 2015)	N/A

Human *SLC7A11* qPCR reverse primer [TCCCAATTCAGCATAAGACAAA]	(Martin and Gardner, 2015)	N/A
Human *GCLM* qPCR forward primer [CATTTACAGCCTTACTGGGAGG]	Primerbank	Accession NM_002061.3
Human *GCLM* qPCR reverse primer [ATGCAGTCAAATCTGGTGGCA]	Primerbank	Accession NM_002061.3
Human *GCLC* qPCR forward primer [GGCGATGAGGTGGAATACAT]	(Rushworth et al., 2011)	N/A
Human *GCLC* qPCR reverse primer [GTCCTTTCCCCCTTCTCTTG]	(Rushworth et al., 2011)	N/A
Human *NQO1* qPCR Forward primer [GCCGCAGACCTTGTGATATT]	(Rushworth et al., 2011)	N/A
Human *NQO1* qPCR Reverse primer [TTTCAGAATGGCAGGGACTC]	(Rushworth et al., 2011)	N/A
Human *HMOX1* qPCR Forward primer [GGCCAGCAACAAAGTGCAAG]	Designed in PrimerBlast	N/A
Human *HMOX1* qPCR Reverse primer [TGGCATAAAGCCCTACAGCA]	Designed in PrimerBlast	N/A
Human *NRF2* qPCR Forward primer [GAGAGCCCAGTCTTCATTGC]	(Reichard et al., 2007)	N/A
Human *NRF2* qPCR Reverse primer [TGCTCAATGTCCTGTTGCAT]	(Reichard et al., 2007)	N/A
Murine *Actb* qPCR forward primer [TCCTAGCACCATGAAGATCAAGATC]	(Brady et al., 2011)	N/A
Murine *Actb* qPCR reverse primer [CTGCTTGCTGAT CCACATCTG]	(Brady et al., 2011)	N/A
Murine *Cdkn1a* qPCR forward primer [CACAGCTCAGTGGACTGGAA]	(Brady et al., 2011)	N/A
Murine *Cdkn1a* qPCR reverse primer [ACCCTAGACCCACAATGCAG]	(Brady et al., 2011)	N/A
Murine *Mdm2* qPCR forward primer [GGACTCGGAAGATTACAGCCTGA]	(Li et al., 2012)	N/A
Murine *Mdm2* qPCR reverse primer [TGTCTGATAGACTGTGACCCG]	(Li et al., 2012)	N/A
TP53 sgRNA #1 Forward Primer [CACCGCCATTGTTCAATATCGTCCG]	CRISPR Design Tool (http://crispr.mit.edu)	N/A
TP53 sgRNA #1 Reverse Primer [AAACCGGACGATATTGAACAATGGC]	CRISPR Design Tool (http://crispr.mit.edu)	N/A
CDKN1A sgRNA #1 Forward Primer [CACCGTACCCTTGTGCCTCGCTCAG]	CRISPR Design Tool (http://crispr.mit.edu)	N/A
CDKN1A sgRNA #1 Reverse Primer [AAACCTGAGCGAGGCACAAGGGTAC]	CRISPR Design Tool (http://crispr.mit.edu)	N/A
Hs_SLC7A11_2 FlexiTube siRNA	Qiagen	Cat# SI00104902
Allstars Negative Control SiRNA	Qiagen	Cat# 1027280
AllStars Hs Cell Death siRNA	Qiagen	Cat# 1027298
Recombinant DNA		
pSpCas9 (BB)-2A-GFP-sgRNA-p53	This paper	N/A
pLenti-CMV-Puro-*CDKN1A*	This paper	N/A
Other Plasmids		
pMD2.G	Addgene	Cat# 12259
psPax2	Addgene	Cat# 12260
pSpCas9 (BB)-2A-GFP	Addgene	Cat# PX458
pDONR223-*CDKN1A*	Human Orfeome V7.1	Clone ID 2821049

pLenti-CMV-Puro DEST	Addgene	Cat# w118-1
pLKO.1 sh*CDKN1A*	Sigma-Aldrich (Mission)	Cat# TRCN000287021
pLKO.1 shSCR	Addgene	Cat# 1864
Software and Algorithms		
Prism	GraphPad Software	N/A
Excel	Microsoft Corp.	N/A
FlowJo	FlowJo LLC.	N/A
Data availability		
N/A		

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