ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway

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It is well established that ferroptosis is primarily controlled by glutathione peroxidase 4 (GPX4). Surprisingly, we observed that p53 activation modulates ferroptotic responses without apparent effects on GPX4 function. Instead, ALOX12 inactivation diminishes p53-mediated ferroptosis induced by reactive oxygen species stress and abrogates p53-dependent inhibition of tumour growth in xenograft models, suggesting that ALOX12 is critical for p53-mediated ferroptosis. The ALOX12 gene resides on human chromosome 17p13.1, a hotspot of monoallelic deletion in human cancers. Loss of one Alox12 allele is sufficient to accelerate tumorigenesis in Eph-Myc lymphoma models. Moreover, ALOX12 missense mutations from human cancers abrogate its ability to oxygenate polyunsaturated fatty acids and to induce p53-mediated ferroptosis. Notably, ALOX12 is dispensable for ferroptosis induced by erastin or GPX4 inhibitors; conversely, ACSL4 is required for ferroptosis upon GPX4 inhibition but dispensable for p53-mediated ferroptosis. Thus, our study identifies an ALOX12-mediated, ACSL4-independent ferroptosis pathway that is critical for p53-dependent tumour suppression.

Although p53-mediated cell-cycle arrest, senescence and apoptosis serve as critical barriers to cancer development, accumulating evidence reveals that p53-mediated metabolic regulation also promotes tumour suppression. We and others have recently found that p53 plays an important role in modulating ferroptotic responses through its metabolic targets. Nevertheless, the molecular factors that mediate p53-dependent ferroptosis have not been delineated and the mechanism by which this ferroptosis pathway is regulated remains unclear. Ferroptosis is a regulated form of cell death driven by excess accumulation of lipid peroxides. Lipid peroxides are normally eliminated by glutathione peroxidase 4 (GPX4) and its co-factor glutathione (GSH), which convert lipid hydroperoxides to non-toxic lipid alcohols. It is well established that ferroptosis is primarily controlled by GPX4. Although inactivation of p53 expression can partially reduce erastin-initiated cell death in certain cell types, the ferroptotic responses induced by erastin or GPX4 inhibitors are not dependent on p53 status. Thus, it remains unclear whether p53-dependent ferroptosis acts through modulation of GPX4 function.

Results
Identification of ALOX12 as an essential factor of p53-dependent ferroptosis. In our previous study, we established a ferroptosis assay that requires both p53 activation and reactive oxygen species (ROS)-induced stress (see Methods). Indeed, the cell death induced by low levels of tert-Butyl hydroperoxide (TBH), a common ROS generator, is apparently p53 dependent and can be specifically inhibited by the ferroptosis inhibitor ferrostatin-1 (Ferr-1), but not by the inhibitors of other cell death pathways such as apoptosis, autophagy or necroptosis. As ferroptotic cell death is tightly regulated in response to oxidative stress in vivo, we reasoned that this type of ferroptosis would better reflect p53 function during an oxidative stress response. In addition to GPX4-mediated neutralization of lipid peroxidation, the levels of cellular lipid peroxides can be induced enzymatically by the lipoxygenases. The mammalian lipoxygenase family consists of six isoforms (ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15 and ALOX15B) with differing substrate specificities. To ascertain whether any of these lipoxygenases are required for p53-mediated ferroptosis, we performed an RNA interference (RNAi)-mediated loss-of-function screen to test whether depletion of individual isoforms affects p53-dependent ferroptosis. Quantitative PCR analyses confirmed that the expression of each of the six lipoxygenase isoforms was individually abrogated by RNAi-mediated depletion (Supplementary Fig. 1a). As expected, high levels of p53-mediated ferroptosis upon ROS-induced stress were detected in TBH-treated p53 knockout H1299 cells transfected with control short interfering RNAs (siRNAs). However, ferroptosis was significantly and specifically blocked by RNAi-mediated depletion of ALOX12, but not by any of the other five lipoxygenases (Fig. 1a). Western blot analysis revealed that ALOX12 depletion had no effect on either p53 levels or p53-mediated downregulation of the cystine/glutamate transporter SLC7A11 (Fig. 1b).

Activation of p53 can be readily induced by Nutlin, a small-molecular inhibitor of MDM2. Although in most human cancer cells, Nutlin-mediated p53 activation induces reversible cell-cycle arrest but not cell death, we previously showed that U2OS cells undergo p53-dependent, ROS stress-induced ferroptosis upon combined treatment with both Nutlin and TBH. Again, although ALOX12 depletion had no obvious effect on p53 levels or expression of its transcriptional targets (for example, SLC7A11, MDM2 and p21) (Fig. 1d), p53-mediated ferroptosis was largely abrogated (Fig. 1c). To further validate the role of ALOX12 in p53-mediated ferroptosis, we used clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) technology
Fig. 1 | ALOX12 is essential for p53-mediated ferroptosis upon ROS stress. a, H1299 Tet-on p53^ΔEx cells transfected with control siRNA (ctrl) or a pool of ALOX family-specific siRNAs pre-incubated with doxycycline (0.5 µg ml⁻¹; Tet) for 12 h, then treated with doxycycline (0.5 µg ml⁻¹) and TBH (40 µM) as indicated for 8 h. Mean ± s.d. is shown; n = 3 independent experiments. b, Western blot analysis of H1299 Tet-on p53^ΔEx cells transfected with control or ALOX12 siRNA (si) and then treated with doxycycline (0.5 µg ml⁻¹) as indicated for 24 h. The experiments were repeated twice, independently, with similar results. c, U2OS cells transfected with control or ALOX12 siRNA were pre-incubated with Nutlin (10 µM) for 12 h, and then the cells were treated with Nutlin (10 µM) and TBH (300 µM) as indicated for 8 h. Mean ± s.d. is shown; n = 3 independent experiments. d, Western blot analysis of U2OS cells transfected with control or ALOX12 siRNA and then treated with Nutlin (10 µM) as indicated for 48 h. The experiments were repeated twice, independently, with similar results. e, Representative phase-contrast images of U2OS ctrl CRISPR (top panels) and ALOX12 CRISPR (bottom panels) cells pre-incubated with Nutlin (10 µM) for 12 h were treated with TBH (300 µM), Nutlin (10 µM) and Ferr-1 (2 µM) as indicated for 8 h. Scale bars, 100 µm. The experiments were repeated twice, independently, with similar results. All P values (a,c,f) were calculated using two-tailed unpaired Student’s t-test. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 9. Raw data are provided in Supplementary Table 1.
to generate ALOX12-knockout subclones of U2OS cells. Indeed, ferroptosis was suppressed in ALOX12-knockout U2OS cells (Fig. 1e,f and Supplementary Fig. 1c–e). Taken together, these data indicate that ALOX12 is required for p53-dependent ferroptosis.

**GPX4 inhibition is not required for p53-mediated ferroptosis upon ROS stress.** Interestingly, a systematic genome-wide screen failed to identify any lipoxigenase enzyme(s) required for ferroptosis induced by GPX4 inhibitors or erastin26. Thus, our finding that ALOX12 is specifically required for p53-mediated ferroptosis suppression is surprising and suggests that p53-mediated ferroptosis may act through a different mechanism. Lipid peroxides are normally eliminated by GPX4 and its co-factor GSH and ferroptosis can be induced by pharmacological agents including GPX4 enzymatic inhibitors and erastin, which induces GSH depletion and consequent GPX4 inactivation. To this end, we first examined the ratio of GSH/GSSG and the levels of GSH upon p53 activation. As shown in Fig. 2a,b, the ratio of reductive GSH to oxidative GSSG and the levels of GSH were dramatically reduced upon erastin treatment; conversely, following p53 activation by treatment with Nutlin, no obvious effect was detected. Consistent with these observations, activation of p53 by Nutlin treatment failed to suppress GSH peroxidase activity (Supplementary Fig. 2a) and had no obvious effect on endogenous lipid peroxidation (Supplementary Fig. 2b,c). Nevertheless, increasing lipid peroxidation levels were detected upon combined treatment with both Nutlin and TBH (Supplementary Fig. 2b,c).

To further support this notion, we examined whether p53 activation has any effect on GPX4-mediated activity of endogenous lipid peroxidation levels. To this end, we first established GPX4-null p53 tetracycline (Tet)-on H1299 cells by CRISPR-mediated knockout (Fig. 2c) and then analyzed the levels of endogenous lipid peroxidation by flow cytometry with C11-BODIPY staining. As shown in Fig. 2d, high levels of endogenous lipid peroxidation were detected in the GPX4-null cells, whereas upon GPX4 expression ectopically, the levels of lipid peroxidation were reduced dramatically (also see Supplementary Fig. 2d,e). Thus, the drastic reduction of lipid peroxidation levels represents the activity of GPX4 on endogenous lipid peroxidation. As expected, treatment with the known GPX4 inhibitor RSL-3 largely abrogated the effects on lipid peroxidation reduction induced by GPX4 in those cells; however, activation of p53 failed to induce any obvious effect (Fig. 2d). Taken together, these data indicate that p53-dependent activation of ferroptosis may act through a distinct pathway, independent of GPX4 modulation.

**Inactivation of ALOX12 abrogates p53-mediated tumour growth suppression.** Using xenograft tumour models in mice, we previously showed that the tumour suppression activity of p53√KR cells, which is defective for the canonical p53 tumour suppression functions (cell-cycle arrest, apoptosis and senescence), is instead dependent on p53-mediated ferroptosis. To ascertain whether ALOX12 is also required for p53-mediated tumour suppression in this setting, we first established isogenic lines of Tet-inducible p53√KR cells in which the ALOX12 gene has or has not been knocked out by CRISPR–Cas9 technology (Supplementary Fig. 3a). Indeed, the levels of ferroptosis were dramatically diminished in ALOX12-knockout cells (Fig. 2e and Supplementary Fig. 3b,c). To examine whether ALOX12 contributes to the tumour suppression activity of p53, we tested whether loss of ALOX12 expression affects tumour cell growth suppression by p53√KR in xenograft tumour models. As expected, Tet-induced expression of p53√KR significantly reduced tumour cell growth in this assay (Fig. 2f and Supplementary Fig. 3d,e); however, the tumour suppression effects of p53√KR were ablated in ALOX12-knockout cells. Notably, upregulation of Ptg2, a marker of ferroptosis18, was also abrogated in ALOX12-knockout cells (Fig. 2g). These data demonstrate that ALOX12 is crucial for the tumour cell growth suppression activity of p53 in the absence of p53-mediated cell-cycle arrest, apoptosis and senescence.

**Loss of one Alox12 allele is sufficient to abrogate p53-mediated ferroptosis and accelerate tumorigenesis.** To further validate the role of ALOX12 in p53-mediated ferroptotic responses, we compared the sensitivity of isogenic ALOX12-mutant mouse embryonic fibroblasts (MEFs) to p53-mediated ferroptosis. As shown in Fig. 3a, ALOX12 protein levels were undetectable in homozygous (Alox12−/−) MEFs and significantly reduced in heterozygous (Alox12+/-) MEFs. As expected, ferroptosis was readily induced by TBH in wild-type, but not in p53-null, MEFs under the same conditions (Fig. 3b,c), indicating that TBH-induced ferroptosis is largely p53-dependent under these conditions. Strikingly, however, cell death was not observed in either Alox12+/- or Alox12−/− MEFs. These data indicate that ALOX12 is haploinsufficient with respect to p53-mediated ferroptosis.

To examine whether ALOX12 also affects tumour suppression in a haploinsufficient manner, we tested whether loss of one Alox12 allele affects Myc-induced tumorigenicity in the classic Eμ-Myc lymphoma model. Previous studies have shown that Eμ-Myc mice develop late-onset B cell lymphomas between 6 and 12 months of age27–30, and that tumour formation is markedly accelerated upon loss of one Trp53 allele (in Eμ-Myc; Trp53−/− mice). Remarkably, we observed that the latency of tumour formation in Eμ-Myc mice (median tumour-free survival of ~220 d) is also dramatically reduced by the absence of one Alox12 allele (Eμ-Myc; Alox12−/− mice; median survival of ~70 d) (Fig. 3d). Of note, Eμ-Myc; Alox12−/− mice presented with severe lymphadenopathy (Fig. 3e) and developed high-grade diffuse B cell lymphomas (Fig. 3f) with a latency similar to that of Eμ-Myc; Trp53−/− mice (median tumour-free survival of ~50 d), indicating that ALOX12-dependent ferroptosis contributes significantly to p53-mediated tumour suppression in the Eμ-Myc lymphoma model.

Although the lymphomas that arise in Eμ-Myc mice often exhibit defects in the ARF–p53 tumour suppression pathway27–29, neither the Arf (also known as Cdkn2a) nor the Trp53 gene was deleted or mutated in tumours derived from Eμ-Myc; Alox12−/− mice (Fig. 3g), which was further validated by sequencing analysis (Supplementary Fig. 4a). Likewise, the major p53 feedback inhibitors, MDM2 and MDMX, were not overexpressed in these tumours (Fig. 3g). Loss of functional Trp53 through the two-hit mechanism is among the most frequent events in human cancers and a ‘loss of heterozygos-
monoallelic loss of ALOX12 impairs both p53-mediated ferroptosis and p53-mediated tumour suppression, the above data indicate that ALOX12 itself functions as a haploinsufficient tumour suppressor.

Consistent with this notion, examination of the Oncomine database reveals that ALOX12 expression is downregulated in various human cancers, including cervical squamous cell carcinoma,
Fig. 3 | Loss of one Alox12 allele is sufficient to accelerate tumorigenesis in Eμ-Myc lymphoma models. a, Western blot analysis of wild-type (WT), Alox12+/− and Alox12−/− MEFs. Two representative MEF cell lines for each genotype are shown. The experiments were repeated twice, independently, with similar results. b, Representative phase-contrast images of WT, Alox12−/−, Alox12−/− and Trp53−/− MEFs treated with TBH (80 μM) as indicated for 12 h. Scale bars, 100 μm. The experiments were repeated three times, independently, with similar results. c, WT (Trp53+/+), Alox12−/− and Trp53−/− MEFs treated with TBH (80 μM) as shown in b. Mean ± s.d. is shown; n = 3 independent experiments. d, Kaplan–Meier survival curves of Eμ-Myc (n = 10 independent mice), Eμ-Myc; Alox12−/− (n = 8 independent mice) and Eμ-Myc; Trp53−/− (n = 12 independent mice). The P value (Eμ-Myc versus Eμ-Myc; Alox12−/− background) was calculated using the log-rank Mantel–Cox test. e, Representative image of an early onset lymphoma in a 45-day-old Eμ-Myc; Alox12−/− mouse. The experiments were repeated three times, independently, with similar results. f, Representative haematoxylin and eosin staining of a high-grade diffuse B cell lymphoma developed from a Eμ-Myc; Alox12−/− mouse. The experiments were repeated three times, independently, with similar results. g, Western blot analysis of Eμ-Myc control samples and tumour samples from Eμ-Myc; Alox12−/− and Eμ-Myc; Trp53−/− mice. The experiments were repeated twice, independently, with similar results. h, Quantitative PCR of Ptgs2 from tumours harvested from Eμ-Myc control samples (n = 3 independent samples) and tumour samples (n = 3 independent samples) from Eμ-Myc; Alox12−/− mice. Mean ± s.d. is shown; n = 3 independent experiments. The P values were calculated using two-tailed unpaired Student’s t-test; P value (Eμ-Myc versus Eμ-Myc; Alox12−/− background) was calculated using the log-rank Mantel–Cox test. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 9. Raw data are provided in Supplementary Table 1.
head and neck squamous cell carcinoma, oesophageal squamous cell carcinoma and acute myeloid leukaemia (Supplementary Fig. 5a,b). In addition, several ALOX12 missense mutations are present in the COSMIC database of human tumour specimens, including R562Q/W (R→Q or W mutation at amino acid 562), A372T/D (A→T or D mutation at amino acid 372) and G381R (G→R mutation at amino acid 381) (Fig. 4a and Supplementary Table 2). As the affected residues are evolutionarily conserved and localized within the lipoxygenase domain (Fig. 4a and Supplementary Fig. 5d), these mutations may have functional consequence. To this end, we first examined the effects of these mutations on lipoxygenase activity. As shown in Fig. 4b, the wild-type ALOX12 protein readily oxidized arachidonic acid in vitro and, as expected, its lipoxygenase activity was inhibited by ML-355, a specific ALOX12 inhibitor25. Moreover, p53-mediated ferroptosis was blocked by ML-355 (Supplementary Fig. 5e,f). Notably, this lipoxygenase activity was significantly impaired by each of the tumour-derived missense mutations tested (G381R, R562Q, R562W, A372T and A372D) (Fig. 4c). Moreover, p53-mediated ferroptosis in ALOX12-null U2OS cells was restored by ectopic expression of wild-type ALOX12 but not by the tumour-derived ALOX12 G381R mutant (Fig. 4d and Supplementary Fig. 5g). Similar data were also obtained in ALOX12-null H1299 cells and further analysis showed that none of these tumour-derived mutants was able to fully induce cell death under the same conditions (Fig. 4c,f). Taken together, these data demonstrate that the ALOX12 gene is downregulated and mutated in human cancers, and that tumour-derived ALOX12 missense mutations are defective in both lipoxygenase activity and the ability to induce p53-mediated ferroptosis.

Mechanistic insight into p53-mediated activation of ALOX12. The specific requirement for ALOX12 in p53-dependent ferroptosis raised the possibility that ALOX12 activity may be regulated by p53. As no obvious effect on ALOX12 levels was detected upon p53 activation (Fig. 1d,g), we examined whether ALOX12 function is modulated by known p53 targets involved in ferroptosis, such as SLC7A11. To this end, we first tested whether SLC7A11 interacts with ALOX12. As shown in Fig. 5a, ALOX12 (lane 4), but not ALOX15 (lane 8), readily co-immunoprecipitated with Flag-haemaggulutinin (HA)-tagged SLC7A11. Indeed, none of other ALOX family members (ALOXE3, ALOX5, ALOX12B and ALOX15B) was detectable in the Flag-SLC7A11 immunoprecipitates (Supplementary Fig. 6a–d), indicating that SLC7A11 interacts specifically with ALOX12. Purified SLC7A11 bound to a recombinant ALOX12 in an in vitro pull-down assay (Fig. 5b), indicating that ALOX12 and SLC7A11 interact directly. Moreover, co-immunoprecipitation analysis confirmed that the endogenous ALOX12 and SLC7A11 polypeptides also interact in vivo (Fig. 5c). Together, these data demonstrate that ALOX12 is a bona fide binding partner of SLC7A11.

To elucidate the functional consequence of this interaction, we tested whether SLC7A11 affects ALOX12 lipoxygenase activity. As shown in Fig. 5d,e, the lipoxygenase activity of ALOX12, but not of ALOX15, was suppressed in a dosage-dependent manner by SLC7A11 expression (Supplementary Fig. 6e,f). Although the protein structure of SLC7A11 has not yet been solved, it is predicted as a 12-pass transmembrane protein with several domains located inside the cell26. Further analyses demonstrate that the intracellular loop of SLC7A11 (amino acids 98–131) plays a key role in binding and repressing ALOX12 lipoxygenase activity (Supplementary Fig. 6h–j). As SLC7A11 levels are downregulated by p53, this observation suggested that p53 can promote ALOX12 function indirectly by downregulating SLC7A11. Indeed, as shown in Fig. 5f, SLC7A11 levels were reduced (right panel) and ALOX12 activities were elevated (left panel) in human U2OS cells upon Nutilin-induced p53 activation. Together, these data demonstrate that (1) SLC7A11 specifically binds to and inhibits the enzymatic activity of ALOX12 and (2) p53 induces ALOX12 lipoxygenase activity by repressing SLC7A11 expression.

ALOX12 is dispensable for ferroptosis induced by erastin or GPX4 inhibitors. To elucidate differences between p53-mediated and erastin-induced ferroptosis, we first examined the effect of erastin on ALOX12 function. Erastin treatment did not affect the interaction between SLC7A11 and ALOX12 (Fig. 5g) or the levels of ALOX12 activity (Fig. 5h). In contrast to p53-mediated ferroptosis, erastin-induced ferroptosis was not significantly affected in isogenic Alox12+/− and Alox12−/− MEFs (Fig. 5i and Supplementary Fig. 7a) or ALOX12-null U2OS cells (Supplementary Fig. 7b,c). Together, these data demonstrate that ALOX12 is not required for erastin-induced ferroptosis.

Thus, although p53 and erastin both induce ferroptosis by targeting the same factor (SLC7A11), the mechanisms are strikingly different, as exemplified by the fact that p53 is ALOX12 dependent, whereas erastin is ALOX12 independent. On the one hand, p53 promotes ferroptosis through transcriptional repression of SLC7A11, which in turn releases the lipoxygenase activity of ALOX12 from SLC7A11 inhibition. On the other hand, although erastin suppresses the antipporter activity of SLC7A11 (ref. 27), it fails to abrogate SLC7A11 inhibition of ALOX12. Nevertheless, erastin treatment ultimately induces ferroptosis by blocking cystine import, GSH biosynthesis and GPX4 activity28. Indeed, most of the described ferroptotic responses, including that induced by erastin, entail inhibition of GPX4 (refs. 29–31). In this regard, we also found that loss of ALOX12 expression has no obvious effect on ferroptosis induced by RSL-3, a specific GPX4 inhibitor (Supplementary Fig. 7d). Thus, the mechanism of p53-dependent ferroptosis is distinct from that of ferroptotic responses induced by erastin or GPX4 inhibitors.

ACSL4 is required for ferroptosis induced by erastin or GPX4 inhibitors but is dispensable for p53-mediated ferroptosis. Recent studies show that the ferroptotic responses induced by either erastin or GPX4 inhibitors are dependent on acyl-CoA synthetase long-chain family member 4 (ACSL4), an enzyme that promotes biosynthesis of unsaturated phospholipids, the main substrates for lipid peroxidation28,29. To further elucidate the mechanism of p53-mediated ferroptosis, we generated ACSL4-knockout cell lines of U2OS (Fig. 6a). As expected, ACSL4-null cells are resistant to ferroptosis induced by either erastin (Fig. 6b) or the GPX4 inhibitor RSL-3 (Fig. 6c). By contrast, however, ACSL4-null cells are fully susceptible to p53-mediated ferroptosis (Fig. 6d). Moreover, we generated Acsl4-mutant mice (Supplementary Fig. 8a–c) and obtained Acsl4−/− MEFs for ferroptotic analysis. As expected, the ACSL4 protein was not detectable in Acsl4−/− MEFs (Fig. 6e) and the levels of ferroptosis induced by erastin (Fig. 6f) or RSL-3 (Fig. 6g) were largely suppressed. Again, however, p53-dependent ferroptosis was fully retained in Acsl4−/− MEFs (Fig. 6h). Together, these data demonstrate that p53-mediated ferroptosis is ALOX12 dependent but ACSL4 independent.

p53-mediated ferroptosis is deregulated in human cancer cell lines expressing wild-type p53. To examine whether p53-mediated ferroptosis is deregulated in human cancers, we examined ALOX12 protein levels in numerous human cancer cell lines that retain wild-type p53. As shown in Fig. 7a, ALOX12 was undetectable in the human fibrosarcoma cell line HT1080, the original cell line characterized for erastin-induced ferroptosis3. As expected, ferroptosis was readily induced in the cells by erastin (Fig. 7c). By contrast, HT1080 cells were not susceptible to p53-mediated ferroptosis (Fig. 7d, left panel), despite the fact that SLC7A11 levels were downregulated upon p53 activation (Fig. 7b). However, these cells were rendered susceptible to p53-mediated ferroptosis by ectopic expression of ALOX12 (Fig. 7d). Similar results were also obtained in the human
**Fig. 4** | ALOX12 missense mutations from human cancers abrogate ALOX12 enzymatic activity and p53-mediated ferroptosis.  

**a,** Schematic diagram of ALOX12 with frequent tumour mutations identified in the lipoxygenase domain identified from the COSMIC database.  

**b,** An in vitro catalytic activity assay of ALOX12 was measured by detecting 12-HETE levels (lipoxygenase activity) by ELISA. Highly purified ALOX12 was incubated with arachidonic acid (AA) and ML-355 (an ALOX12 inhibitor) as indicated (see Methods). Mean ± s.d. is shown; n = 3 independent experiments.  

**c,** An in vitro catalytic activity assay of ALOX12, as described in b, using the indicated ALOX12 tumour mutants as shown in a (top panel). Mean ± s.d. is shown; n = 3 independent experiments. Western blot analysis of highly purified ALOX12 proteins is also shown (bottom panel). The western blot experiments were repeated twice, independently, with similar results.  

**d,** Western blot analysis of H1299 Tet-on p53<sup>338R</sup> ALOX12 CRISPR cells transfected with control and ALOX12 WT or mutant vectors as indicated. The experiments were repeated twice, independently, with similar results.  

**e,** Representative phase-contrast images of H1299 Tet-on p53<sup>338R</sup> ALOX12 CRISPR cells transfected with control (top panels), ALOX12 (middle panels) or mutant ALOX12 G381R (bottom panels) vectors. Cells were pre-incubated with doxycycline (0.5 µg ml<sup>−1</sup>) for 12 h, then treated with doxycycline (0.5 µg ml<sup>−1</sup>) and TBH (60 µM) as indicated for 8 h. Scale bars, 100 µm. The experiments were repeated twice, independently, with similar results.  

**f,** The cell death of H1299 Tet-on p53<sup>338R</sup> ALOX12 CRISPR cells transfected with control or ALOX12 vectors from e as indicated. Mean ± s.d. is shown; n = 3 independent experiments. All P values (b,c,f) were calculated using two-tailed unpaired Student’s t-test. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 9. Raw data are provided in Supplementary Table 1.
Fig. 5 | Mechanistic insight into p53-mediated activation of ALOX12. a, Western blot analysis of the interaction among SLC7A11, ALOX12, and ALOX15. 293T cells were co-transfected with the indicated constructs, and the extract was analysed by co-immunoprecipitation (IP) assays. The experiments were repeated twice, independently, with similar results. b, An in vitro binding assay between purified SLC7A11 and ALOX12. The experiments were repeated twice, independently, with similar results. SFB is S protein tag, Flag tag, Streptavidin-binding peptide. FH-SLC7A11, Flag-HA-tagged SLC7A11. c, Western blot analysis of the endogenous interaction between SLC7A11 and ALOX12 in H1299 cells. The experiments were repeated twice, independently, with similar results. d, An in vivo catalytic activity assay of ALOX12 was measured by detecting 12-HETE levels by ELISA. U2OS cells were co-transfected with SLC7A11 and ALOX12. Mean ± s.d. is shown; n = 3 independent experiments. e, An in vivo catalytic activity assay of ALOX15 was measured by detecting 15-HETE levels by ELISA. U2OS cells were co-transfected with SLC7A11 and ALOX15. Mean ± s.d. is shown; n = 3 independent experiments. f, An in vivo catalytic activity assay of ALOX12 was measured by detecting 12-HETE levels by ELISA (left panel). U2OS cells were transfected with ALOX12 with or without Nutlin (10 µM) treatment. Mean ± s.d. is shown; n = 3 independent experiments. Western blot analysis of U2OS cells with or without Nutlin treatment is also shown (right panel). g, Western blot analysis of the interaction between SLC7A11 and ALOX12 with or without erastin (5 µM) treatment. The experiments were repeated twice, independently, with similar results. h, An in vivo catalytic activity assay of ALOX12 was measured by detecting 12-HETE levels by ELISA. U2OS cells were transfected with ALOX12 with or without erastin (5 µM) treatment. Mean ± s.d. is shown; n = 3 independent experiments. i, WT, Alox12+/− and Alox12−/− MEFs were treated with erastin (10 µM) and Ferr-1 (2 µM) for 12 h. Mean ± s.d. is shown; n = 3 independent experiments. All P values (d–f, h) were calculated using two-tailed unpaired Student’s t-test. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 9. Raw data are provided in Supplementary Table 1.
Fig. 6 | p53-mediated ferroptosis is ALOX12 dependent but ACSL4 independent. a, Western blot analysis of U2OS control CRISPR and ACSL4 CRISPR cells. The experiments were repeated twice, independently, with similar results. b, U2OS control and ACSL4 CRISPR cells treated with erastin (20 µM) and Ferr-1 (2 µM) as indicated for 12 h. Mean ± s.d. is shown; n = 3 independent experiments. c, U2OS control and ACSL4 CRISPR cells treated with RSL-3 (4 µM) and Ferr-1 (2 µM) as indicated for 12 h. Mean ± s.d. is shown, n = 3 independent experiments. d, U2OS control and ACSL4 CRISPR cells pre-incubated with Nutlin (10 µM) for 12 h were treated with TBH (300 µM), Nutlin (10 µM) and Ferr-1 (2 µM) as indicated for 12 h. Mean ± s.d. is shown; n = 3 independent experiments. e, Western blot analysis of WT and Acsl4−/− MEFs. The experiments were repeated twice, independently, with similar results. f, WT and Acsl4−/− MEFs treated with erastin (10 µM) and Ferr-1 (2 µM) as indicated for 9 h. Quantification of cell death is shown; mean ± s.d.; n = 3 independent experiments, except for ctrl n = 9. g, WT and Acsl4−/− MEFs treated with RSL-3 (1 µM) and Ferr-1 (2 µM) as indicated for 9 h. Quantification of cell death is shown; mean ± s.d.; n = 3 independent experiments. h, WT and Acsl4−/− MEFs pre-incubated with Nutlin (10 µM) for 12 h were treated with TBH (80 µM) and Ferr-1 (2 µM) as indicated for 9 h. Quantification of cell death is shown; mean ± s.d.; n = 3 independent experiments. All P values (b–d, f–h) were calculated using two-tailed unpaired Student’s t-test. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 9. Raw data are provided in Supplementary Table 1.

colorectal cancer cell line HCT116 (Fig. 7c, left panel). Of note, it was recently reported that p53-induced ferroptosis was not observed in HCT116 and HT1080 cell lines; instead, p53 stabilization led to repression of erastin-induced ferroptosis in these cells, further validating the specific role of ALOX12 in p53-mediated ferroptosis. Notably, ACSL4 is not expressed in the human breast cancer cell
line MCF-7 (Fig. 7a, lane 7) and, indeed, these cells are resistant to both erastin-mediated (Fig. 8a) and RSL-3-mediated (Fig. 8b) ferroptosis. Nonetheless MCF-7 cells are clearly susceptible to p53-mediated ferroptosis induced by ROS stress (Fig. 8c). Moreover, p53-mediated ferroptosis was also readily detected in other cell lines in which both p53 and ALOX12 are expressed, including the human lung carcinoma lines A549 and H460 and the human melanoma line A375 (Fig. 8d–f). As expected, p53-mediated ferroptosis in these cells was completely suppressed by the ALOX inhibitor ML-355 (Fig. 8d–f), further validating the specific requirement for ALOX12 in p53-mediated ferroptosis.

**Discussion**

Although the precise mechanisms by which p53 induces ferroptosis still require further elucidation, our study emphasizes the importance of ROS in this process. In addition to treatment with TBH, p53-mediated ferroptosis can also be induced by other forms of ROS, such as low levels of hydrogen peroxide (H₂O₂) and paraquat (Fig. 8g and Supplementary Fig. 8d). Thus, p53 promotes tumour suppression at least in part by sensitizing cancer cells to ferroptotic death upon oxidative stress. As high levels of cell proliferation are generally accompanied by increased ROS production, cancer cells are likely to select for various strategies, such as SLC7A11
overexpression or ALOX12 inactivation, to protect themselves from ROS-induced ferroptosis.

The ALOX12 gene resides on human chromosome 17p13.1 at a position very close to the TP53 locus (Supplementary Fig. 8e). As loss of one copy of chromosome 17p13.1 is very common in human cancer, it is likely that many human tumours have lost one allele of ALOX12 (refs. 24–26). Interestingly, recent studies indicate that a substantial fraction (31–36%) of human tumours containing chromosome 17p deletion retain a wild-type TP53 allele24. In light of our data that ALOX12 haploinsufficiency inhibits p53-mediated ferroptosis and accelerates Myc-induced tumorigenesis without obvious alterations in the ARF–p53 pathway, it
is conceivable that monoallelic loss of ALOX12 is an aetiological factor in this subset of patients. Notably, in contrast to the severe phenotypes associated with GPX4-mutant mice, deletion of the Alox12 gene does not elicit major developmental defects in mice15. Thus, ALOX12, in a manner reminiscent of p53, is largely dispensable for most developmental processes that entail ferroptosis. As ALOX12 is deleted, downregulated and mutated in human cancers, the p53–ALOX12 axis may act as a potential barrier to cancer development.

Although numerous studies implicate GPX4 inhibition as a central node for the induction of ferroptosis, our data identify a distinct mechanism through which p53 induces the ferroptotic response. In particular, we found that p53 is able to activate ALOX12 function indirectly by transcriptional repression of SLC7A11, resulting in ALOX12-dependent ferroptosis upon ROS stress (Fig. 8h). Further investigations are needed to examine whether other metabolic targets of p53 also contribute to ALOX12 activation49. Notably, in contrast to the effects by erastin treatment, we observed that p53 activation alone by Nutlin treatment does not significantly induce downregulation of the GSH/GSSG ratio, GSH levels and GPX4 activity in human cancer cells (Fig. 2a,b,d). Although downregulation of SLC7A11 induced by p53 is able to partially inhibit cystine uptake5, the overall effects on the GSH/GSSG ratio and GSH levels are apparently neutralized, probably by activation of other p53 targets, such as TIGAR (TP53-inducible glycolysis and apoptosis regulator) and p21. For example, activation of TIGAR by p53 was found to reduce ROS levels and increase the GSH/GSSG ratio5; p53-mediated activation of p21 was reported to promote the conservation of GSH15. Our data have further demonstrated that ALOX12-mediated ferroptosis is independent of ACSL4 (Fig. 8h). As ACSL4 is required for ferroptosis induced by erastin or GPX4 inhibitors33,35, our study reveals a previously uncovered ferroptotic pathway, distinct from the current ferroptosis model mainly focusing on GPX4 modulation.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41556-019-0305-6.

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Author contributions
B.C. and W.G. conceived the study and experimental design. B.C., N.K., D.C., T. Liu, S.S., T. Li and L.J. conducted the experiments and acquired the data. B.C., O.T. and W.G. analysed and interpreted the data. B.C. and W.G. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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CRISPR–Cas9-knockout U2OS and H1299 Tet-on p533KR cells were generated by transfecting U2OS cells with a mixture of ALOX12 and ALOX12B vectors, and then selecting for puromycin resistance. ALOX12-knockout single clones were then screened and used to generate tet-on p533KR cells. To generate the ALOX12 stable cell line, wild-type or mutant pci4-Flag-HA ALOX12 was transfected into U2OS cells, followed by selection and maintenance with 1 mg ml⁻¹ G418 (Sigma) in DMEM medium containing 10% FBS. Single clones were selected and screened by western blot.

Plasmids. The pcDNA-ALOX12 plasmid was generously gifted from C. D. Funk (Queen’s University, Kingston, Ontario, Canada). For wild-type ALOX12, full-length ALOX12 was subcloned into the pcDNA3.1-V5-His-Topo vector (Invitrogen), and for ALOX12 Topo plasmids were constructed using the QuickChange XL Site-Directed Mutagenesis Kit (Agilent) according to the standard protocol. For Flag-HA wild-type and mutant ALOX12, full-length ALOX12 was subcloned into the pc4-Flag-HA vector. For Flag-HA-SLC7A11, full-length SLC7A11 was subcloned into the pc4-Flag-HA vector. For V5 ALOX12, ALOX12, ALOX12B, ALOX15 and ALOX15B, full-length ALOXs were subcloned into the pcDNA3.1-V5-His-Topo vector.

Western blotting and antibodies. Protein extracts were analysed by western blotting according to standard protocols using primary antibodies against p53 (DO-1; sc-126; Santa Cruz; 1:1,000 dilution), Trp53 (Regisatin, Inc.; 1:1000); for GAPDH, mouse polyclonal antibody (BCF1; sc-11480; Santa Cruz; 1:1000 dilution), and the common primary antibody (48111; BD Biosciences; 1:1000 dilution). To detect GSH levels, GSH standard solution was used to prepare the standard curve (1 mg ml⁻¹ enzyme/coenzyme working solution, incubated at 37 °C for 10 min and the plate was read at 405 nm.

Expression, purification and activity assay of ALOX12 in vitro. For purification of ALOX12 proteins from U2OS cells, U2OS cells were transfected with pcIN4-Flag-HA-AOX12 and grown for 24 h. Then, the cells were harvested and lysed using BC100 buffer (20 mM Tris-HCl pH 7.3, 100 mM NaCl, 1% Triton X-100, 10 mM EDTA, 1% NP-40 and proteinase inhibitor, with a final concentration of 0.1 mM TBP (triphenylphosphine) on ice for 1 h. The cell lysate was centrifuged for 15 min at 15,000 r.p.m. at 4 °C. The supernatants were used for Flag/2M immunoprecipitations by anti-Flag antibody-conjugated agarose (Sigma) and incubated overnight. The bound ALOX12 proteins were eluted with 10 μg ml⁻¹ Flag peptide (Sigma) in BC100 solution.

Conclusion. By carefully choosing a substrate of AOX12, active AOX12 will metabolize arachidonic acid into 12-hydroxyeicosatetraenoic acid (12-HETE). Specifically, active AOX12 reduces 12(S)-hydroxyprostanoic acid (12(S)-HPETE) into 12-HETE. The amount of 12-HETE can be measured by enzyme-linked immunosorbent assay (ELISA). Thus, for the AOX12 catalytic activity assay, aliquots of the purified AOX12 enzyme (500 ng) were incubated with arachidonic acid (100 μM) for 10 min in PBS containing 0.1 mM ATP (New England Biosabs) and 0.4 mM Ca²⁺ with or without 4 μM ML-355 (as in Fig. 4b). Then, the reaction solution was acidified with acetic acid to a pH of approximately 3–4 as determined by standard pH paper. The samples were extracted with equal volume ethyl acetate and then dried and dissolved in 20 μl ethanol. To detect 12-HETE levels, we performed a 12-HETE ELISA. Purified 12-HETE (100 μg) was used to generate a serial dilution for the standard curve (1 mg ml⁻¹; Detroit R&D). The samples from the purified AOX12 reaction buffer were diluted to 500 μl with a 1x sample dilution buffer.

Drugs and inhibitors. For ROS generation, TRH (Sigma) was used at different doses depending on the experiment; see respective figure legends. A specific GPX4 inhibitor (B6095; APEXBio) was used. A specific cell death inhibitor and AOX12 activity inhibitor was used in the experiments: Fer-1 (ferroptosis inhibitor; Xcess Biosciences) and ML-355 (AOX12 inhibitor; Cayman Chemical) were used at different doses depending on the experiment; see respective figure legends.

Measurement of GSH in vivo. To measure total GSH and the ratio of reductive GSH to oxidative GSH, we used the GSSG/GSH quantification kit (Dojindo Molecular Technologies). U2OS cells were collected and washed with PBS. The cells were lysed by freezing and thawing twice upon the addition of 10 mM HCl. The cells were then added in 5% 5-sulfosalicylic acid and centrifuged at 8,000g for 10 min. The supernatants with a final concentration of 0.5% 5-sulfosalicylic acid were used for the assays. To detect GSH levels, GSH standard solution was used to generate a serial dilution for the standard curve (200 μM).

Measurement of GPX4 activity in vivo. To measure GPX4 activity, we used the GSH peroxidase assay kit (Abcam). U2OS cells transfected with exogenous GPX4 were harvested and washed with cold PBS. The cells were lysed with 200 μl...
The wild-type 3′ primer TCGCGTGGCACACACTAAGTGCTA; and the knockout 5′
primer ATCCGGTGGCCAGACATTCTAAA.

**ALOX12 gene expression analysis.** The ALOX12 gene expression data for normal
cancer versus cancer human tissues were derived from the Oncomine database (https://
www.oncomine.org/resource/). Box plots were derived from gene expression data
in Oncomine comparing the expression of ALOX12 mRNA in different cancer
types. For cervical normal tissues: \( n = 10 \) independent samples, tumour tissues:
\( n = 21 \) independent samples; head and neck normal tissues: \( n = 13 \) independent
samples, tumour tissues: \( n = 41 \) independent samples; oesophageal normal tissues:
\( n = 17 \) independent samples, tumour tissues: \( n = 17 \) independent samples; acute
myeloid leukaemia normal tissues: \( n = 8 \) independent samples, tumour tissues:
\( n = 285 \) independent samples. For box plots, the centre line indicates the median,
the box limits are the 25th and 75th percentiles. The association of ALOX12
with overall survival in patients with pancreatic adenocarcinoma were derived
from publicly available clinical information provided by cbioportal for Cancer
Genomics databases (http://www.cbioportal.org/). Kaplan–Meier survival curves
were generated for overall survival (months) by stratifying patient samples with
pancreatic adenocarcinoma (The Cancer Genome Atlas (TCGA), provisional)
from cbioPortal (\( n = 186 \)) based on ALOX12 expression levels. The patients were
quartiled for ALOX12 expression (ALOX12 low (\( n = 45 \)) black and ALOX12 high
(\( n = 45 \)) red) and the log-rank Mantel–Cox test was used (\( P = 0.016 \)).

**Statistics and reproducibility.** Figures 1b,d,e,g, 2c,f, 3a,g, 4c(bottom panel),
d,e, 5a–c (right panel)g, 6a,e and 7a,b.d (right panel), e (right panel) and
Supplementary Figs. 1c, 3a,e, 4a–e, 6a–f,h–i and 8b,c were independently repeated
twice. All of the other experiments were independently repeated at least three
times. Statistical analysis was carried out using Microsoft Excel software and
GraphPad Prism to assess the differences between experimental groups. Statistical
significance was determined by using a two-tailed, unpaired Student’s \( t \)-test with
a confidence interval of 95%. \( P \leq 0.05 \) was denoted as statistically significant.
Statistical analysis of all survival curves data was performed using the log-rank
(Mantel–Cox) test.

**Reporting Summary.** Further information on research design is available in the
Nature Research Reporting Summary linked to this article.

**Data availability**

The ALOX12 gene expression data for normal versus cancer human tissues were
derived from the Oncomine database (https://www.oncomine.org/resource/).
The association of ALOX12 with overall survival in patients with pancreatic
adenocarcinoma were derived from publicly available clinical information provided
by cbioportal for Cancer Genomics databases (http://www.cbioportal.org/).
Source data for Figs. 1a.c,f, 2a,b,d,e.g, 3c,d,b, 4b,c,f, 5d–f,h,i, 6b–d,f,h, 7a–c and 8d have been
derived as Supplementary Table 1. All other data supporting the findings of this
study are available from the corresponding author on reasonable request.

**Code availability**

No computational code was used in this study.

**References**

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hydroperoxides with rat liver microsomal fractions. Biochem. J. 257, 
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  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Cell death was recorded with the Countess II (Life Technologies); Becton Dickinson FACSCalibur machine was used to collect flow cytometry data. ALOX12 and GPX4 enzymatic activity assay and Glutathione levels were recorded with a 96-wells plate reader (BIO-Tek Instruments, INC).

Data analysis

Data quantification were performed with Excel, Image J and CellQuest and statistical analysis with Excel and GraphPad Prism V6. Images were processed with Adobe Photoshop.

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The ALOX12 gene expression data for normal versus cancer human tissues were derived from Oncomine database (https://www.oncomine.org/resource/). Source data for Figs. 1a,c,f, 2a-b, d-e, g, 3c-d, h, 4b-c,f, 5d-f,h-i, 6b-d,f-h, 7c-e and 8a-g and Supplementary Figs. 1b,d,e, 2b, 3b-d, 5c-d, 6j, 7a-c and 8d have been provided as Supplementary Table 1. All other data supporting the findings of this study are available from the corresponding author on reasonable request.
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Life sciences study design

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Sample size  The sample size was determined based on our experiments. For animal experiment (survival curve in Fig. 3d) has at least n=6 independent mice per group. Unless explicitly stated, 3 independent experiments were performed to achieve Student’s t-test analysis.

Data exclusions  No data was excluded from the study.

Replication  Figs. 1b,d,e,g, 2c,f, 3a,g, 4c(bottom panel), d-e, 5a-c,f(right panel),g, 6a,e, 7a-b, d(right panel),e(right panel) and Supplementary Figs. 1c, 3a,e, 4a-c, 6a-f,h,i, 8b-c were independently repeated twice. All the other experiments were independently repeated at least three times.

Randomization  6-8 week female nude mice were chosen as xenograft models, and randomly allocated into experimental groups.

Blinding  For in vitro cell-based experiments, the investigators were not blinded during data acquisition and analysis. The application of treatments and processing procedures made it difficult for blinding but there was no human bias given all the data were collected independently using instrumentation. For the animal experiments the investigators were not blinded to the group allocation. However, at least two observers measured xenograft tumor volumes/weights or the latency of tumor formation/survival time to alleviate human bias in these data.

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| ☐ Eukaryotic cell lines         | ☐ Flow cytometry |
| ☐ Palaeontology                 | ☐ MRI-based neuroimaging |
| ☐ Animals and other organisms   |         |
| ☐ Human research participants   |         |
| ☐ Clinical data                 |         |

Antibodies

Primary antibodies specific for p53 (DO-1) (SC-126 ; Santa Cruz;1:1000 dilution), p53 (FL-393) (SC-6243 ; Santa Cruz;1:1000 dilution), MDM2 (Ab-5) Mouse mAb (4B2C1.11)(OP-145; Millipore; 1:100 dilution), p21 (SX118) (sc-53870; Santa Cruz; 1:1000 dilution), MDMX (A300-287A; Bethyl; 1:1000 dilution), p19-ARF antibody (SC-3) (ab-26696; Abcam; 1:1000 dilution), PUMA antibody (H-136) (sc-28226; Santa Cruz; 1:500 dilution), SLC7A11 antibody (D2M7A) (12691s; Cell Signaling; 1:500 dilution), ACSL4 antibody (AS) (sc-271800; Santa Cruz; 1:1000 dilution), V5 (R960-25; Invitrogen; 1:1000 dilution), HA (118 67423001; Sigma; 1:1000 dilution), ALOX12 antibody (C-5) (sc-365194; Santa Cruz; 1:200 dilution), ALOX15 (ab-80221; Abcam; 1:1000 dilution) and vinculin (V9264; Sigma-Aldrich; 1:5000 dilution). The more detailed information is provided in the Supplementary Table 3.

Validation  All antibodies used were validated by the respective commercial source for the application used .
p53( DO-1), https://www.scbt.com/scbt/product/p53-antibody-do-1;
p53(FL-393), https://www.scbt.com/scbt/product/p53-antibody-fl-393;
MDM2, http://www.emdmillipore.com/US/en/product/Anti-MDM2-Ab-5-Mouse-mAb-4B2C1.11,EMD_BIO-OP145;
p21, https://www.scbt.com/scbt/product/p21-antibody-sx118;
MDMX, https://www.bethyl.com/product/A300-287A/HdmX+MDM4+Antibody;
p19-ARF, https://www.abcam.com/cdkn2ap19arf-antibody-5-c3-ab26696.html;
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V5, https://www.thermofisher.com/antibody/product/V5-Tag-Antibody-Monoclonal/R960-25;
HA, https://www.sigmaaldrich.com/catalog/product/roche/roahaha?lang=en&region=US,
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

U2OS, H1299, A549, A375, MCF7, HCT116, HT1080, H460 and SISA cancer lines were obtained from American Type Culture Collection (ATCC) and have been proven to be negative for mycoplasma contamination. Mouse embryonic fibroblasts (MEFs) were generated from day 13.5 embryos.

Authentication

The cell lines were not authenticated.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines

No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI biosample.

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

Laboratory animals

\( \mu \)-Myc, Trp53+/- and ALOX12-/- mice with both male and female for breeding at 6-8 weeks were ordered from Jackson Laboratories. \( \mu \)-myc, \( \mu \)-myc; ALOX12+/-, and \( \mu \)-myc; p53+/- with both gender from 1-12 months of age were used to determine tumorigenesis and survival rate. For ACSL4-/- mice, gene targeting of mouse ACSL4 gene was designed to flank the second coding exon (exon 2) with loxP sites (flox) to generate a conditional knockout allele of ACSL4. The cre mediated deletion of exon 2 causes translational reading frame shift and truncation of the rest of the ACSL4 protein. The ACSL4 conditional knockout mice were maintained on a mixed background of 129Sv and C57BL/6J through breeding between littermates. NU/J nude female mice at 6-8 week old purchased from Charles River were used for xenograft model.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

The study is compliant with all relevant ethical regulations for animal experiments. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of Columbia University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

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- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

The cells were incubated with medium containing 5uM of BODIPY-C11 dye and the cells were returned to the cell culture incubator for 20 min. Cells were harvested and washed two times with PBS followed by re-suspending in 500ul of PBS.

Instrument

Becton Dickinson FACSCalibur machine

Software

Using CellQuest to collect data

Cell population abundance

10,000 cells were analyzed for each sample

Gating strategy

The starting cell population gating by Forward Scatter and Side Scatter was used to make sure doublet exclusion. Only single cell was used for analysis.

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