Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death

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Cholesterol is essential for cells to grow and proliferate. Normal mammalian cells meet their need for cholesterol through its uptake or de novo synthesis1, but the extent to which cancer cells rely on each of these pathways remains poorly understood. Here, using a competitive proliferation assay on a pooled collection of DNA-barcoded cell lines, we identify a subset of cancer cells that is auxotrophic for cholesterol and thus highly dependent on its uptake. Through metabolic gene expression analysis, we pinpoint the loss of squalene monoxygenase expression as a cause of cholesterol auxotrophy, particularly in ALK+ anaplastic large cell lymphoma (ALCL) cell lines and primary tumours. Squalene monoxygenase catalyses the oxidation of squalene to 2,3-oxidosqualene in the cholesterol synthesis pathway and its loss results in accumulation of the upstream metabolite squalene, which is normally undetectable. In ALK+ ALCCLs, squalene alters the cellular lipid profile and protects cancer cells from ferroptotic cell death, providing a growth advantage under conditions of oxidative stress and in tumour xenografts. Finally, a CRISPR-based genetic screen identified cholesterol uptake by the low-density lipoprotein receptor as essential for the growth of ALCL cells in culture and as patient-derived xenografts. This work reveals that the cholesterol auxotrophy of ALCCLs is a targetable liability and, more broadly, that systematic approaches can be used to identify nutrient dependencies unique to individual cancer types.

Cancer cells can be auxotrophic for specific nutrients owing to mutations or decreased expression of metabolic genes.3,4 The resulting nutrient dependencies provide potential anti-cancer therapies, with the treatment of leukaemia with l-asparaginase as the clearest example. Beyond conferring a nutrient dependency, loss of the activity of a metabolic enzyme can also have marked effects on the levels of intermediate metabolites, which may in turn affect non-metabolic cellular processes.3,6 Therefore, the identification of cancer nutrient auxotrophies can both inform the development of future therapies and elucidate secondary roles for metabolites.

Cholesterol is a cell-non-essential nutrient because, in addition to being taken up from the environment, it can be synthesized de novo from acetyl-coenzyme A (acetyl-CoA) (Fig. 1a). Although cholesterol auxotrophy is an exceedingly rare phenotypic trait in normal diploid cells,7,8 some cancer cell lines are known to depend on exogenous cholesterol for their growth. For example, the histiocytic lymphoma cell line U-937 is cholesterol auxotrophic owing to a defect in 3-ketosteroid reductase (HSD17B7)7. When incubated in a lipoprotein-depleted serum, U-937 cells die after four days unless supplemented with cholesterol (Fig. 1b, Extended Data Fig. 1a). To determine whether similar cholesterol dependencies are observed in additional cancer cell lines, we undertook a competitive proliferation assay with a pooled collection of 28 cancer cell lines, each marked with a lentiviral-transduced DNA barcode (Fig. 1c). As lipoproteins are the major carriers of cholesterol in human serum,9 the pooled population of cell lines was cultured in lipoprotein-replete or lipoprotein-depleted medium for three weeks. Of note, the absence of lipoproteins did not affect the proliferation of most cell lines, suggesting that they can obtain sufficient cholesterol through de novo synthesis (Fig. 1d, Extended Data Fig. 1b, c, Supplementary Table 1). However, lipoprotein depletion did strongly reduce the proliferation of a subset of cancer cell lines, not only U-937, but also U266B1, Raji, Jiyoye and SNU-1—cell lines not previously known to be sensitive to lipoprotein depletion (Fig. 1d, e). In individual growth assays, these cells can proliferate in lipoprotein-depleted conditions when supplemented with free cholesterol, arguing against an essential role for other components of lipoproteins (Extended Data Fig. 1c, d). These data demonstrate that strong extracellular cholesterol dependencies can exist in cancer cells of distinct origins.

Cholesterol biosynthesis occurs through a pathway of over thirty successive steps that converts acetyl-CoA to squalene, which is then cyclized into lanosterol and other downstream sterol compounds. Although oncogenic alterations may reprogram cholesterol metabolism in a subset of cancers10, the presence or absence of such mutations did not correlate with differences in the sensitivity to cholesterol depletion (Extended Data Fig. 1e). We therefore considered that a defect in a cholesterol synthesis gene might render these cells auxotrophic for cholesterol. Analysis of transcriptome-wide gene expression data from the Cancer Cell Line Encyclopedia (CCLE) revealed that one of the cholesterol-dependent cell lines, SNU-1, does not express squalene monoxygenase mRNA or SQLE protein (Fig. 2a, Extended Data Fig. 1f, g). Consistent with a block in the SQLE-catalysed step of cholesterol biosynthesis, expression of SQLE was sufficient to enable the proliferation of SNU-1 cells under cholesterol-depleted conditions (Fig. 2b, Extended Data Fig. 1g). SQLE catalyses the conversion of squalene to squalene-2,3-epoxide and is a rate-liming step in sterol synthesis in mammalian cells (Extended Data Fig. 1f). Although squalene, the upstream metabolite in this reaction, is undetectable in most cancer and normal cells, lack of SQLE expression results in accumulation of squalene in SNU-1 cells, which further validates the absence of SQLE activity (Fig. 2c). Thus, the SNU-1 cell line is a bona fide cholesterol auxotroph because of its lack of SQLE activity.

Using gene expression data from the CCLE, we identified nine additional cell lines without detectable SQLE mRNA and SQLE protein. Of note, six of these cell lines (SU-DHL-1, KIK, SUP-M2, DEL, SR-786 and Karpas299) belong specifically to the cancer subtype ALK+ ALCCLs (Fig. 2d, e). Similar to SNU-1, SQLE-deficient ALCCL cell lines are sensitive to cholesterol depletion (Fig. 2f) and accumulate high amounts of squalene (Fig. 2g). Chemical imaging using stimulated Raman scattering (SRS) microscopy further revealed that, whereas control cells lack squalene, ALCCLs accumulate it in lipid droplets (Fig. 2h, Extended...
a Negative selection screen to identify genes the loss of which inhibits the fitness of cholesterol auxotrophic ALK\(^+\) ALCL cells but not of their SQLE-expressing counterparts or a prototrophic cell line (Fig. 3a, Extended Data Fig. 4a). The highest scoring gene in these screens was the low-density lipoprotein (LDL) receptor (LDLR) (Fig. 3b, Extended Data Fig. 4b–d, Supplementary Table 4). LDLR provides a major source of cholesterol for mammalian cells by serving as the cell-surface receptor for the endocytosis of cholesterol-rich LDL\(^1^9\). Consistent with the screen results and the essential role of LDLR in the survival of ALCLs, depletion of LDLR using a conditional CRISPR–Cas9 system (Fig. 3c, Extended Data Fig. 4e–i) or targeting it with a monoclonal anti-LDLR antibody (Extended Data Fig. 4j–l) strongly decreased the proliferation of ALK\(^+\) ALCL lines but not of control cells. ALK\(^+\) ALCLs upregulate LDLR-mediated cholesterol uptake (Extended Data Fig. 5a) and expression of cholesterol uptake genes (Fig. 3d, Extended Data Fig. 5b–e) to compensate for their deficiency in de novo cholesterol biosynthesis, an adaptation essential for ALK\(^+\) ALCL cells to proliferate. Consistent with these findings, CRISPR–Cas9-mediated LDLR depletion inhibited the growth of mouse tumour xenografts derived from ALK\(^+\) ALCL cancer cell lines (DEL and Karpas299) but not that of a control cell line (KMS-26) (Fig. 3e). To translate our findings to a more relevant in vivo model, we asked whether targeting LDLR affects the growth of PDXs. For this, we performed an in vivo loss-of-function competition assay using a pool of single guide RNAs (sgRNAs) targeting control genomic regions or the LDLR gene. The sgRNAs targeting the LDLR gene strongly inhibited the growth of tumours derived from the DEL cell line as well as from three different ALK\(^+\) ALCL PDXs, but not that of isogenic tumours expressing SQLE (Fig. 3f). Collectively, our data identify cholesterol uptake through LDLR as a therapeutic target for ALK\(^+\) ALCLs in vivo.

We reasoned that a decrease in cholesterol synthesis was unlikely to confer an advantage to cancer cells and so investigated whether loss of SQLE activity might have other beneficial effects on ALCL metabolism and growth. As the accumulation of certain metabolites in cancer cells can promote tumorigenesis by altering cellular processes distinct from the original metabolic pathways in question\(^9\),\(^1^1\),\(^1^3\), we focused our attention on squalene, a relatively poorly characterized metabolic intermediate that accumulates at very high levels upon SQLE loss (Fig. 2g). Squalene is a main component of human sebum and is proposed to have a role as an emollient and a natural antioxidant for the skin, but the role of squalene in cancer biology has been poorly explored\(^1^1\),\(^1^2\). To understand the metabolic consequences of squalene accumulation in ALCLs, we genetically targeted squalene synthase (FDFT1), the enzyme immediately upstream of SQLE in cholesterol biosynthesis responsible for the synthesis of squalene. CRISPR–Cas9-mediated knockout of FDFT1 blocked squalene synthesis, and returned squalene to levels seen in non-ALCL cells (Fig. 4a, b, Extended Data Fig. 6a, b). To test whether squalene accumulation in ALK\(^+\) lymphomas is beneficial for tumour formation in vivo, we generated tumours by injecting FDFT1-knockout Karpas299 cells expressing a vector or an sgRNA-resistant FDFT1 cDNA subcutaneously into immunodeficient mice. Loss of FDFT1 caused a marked decrease in the size of Karpas299 tumours (Fig. 4c). Similarly, competition experiments using FDFT1 and control sgRNAs in two ALK\(^+\) PDX models showed a significant depletion of FDFT1 sgRNAs (Extended Data Fig. 6c). These data suggest that squalene synthesis may be beneficial for optimal growth of ALK\(^+\) ALCLs in vivo.

We next investigated how squalene may affect cellular metabolism in ALK\(^+\) ALCLs. As accumulation of lipid peroxides are detrimental to cell viability, most mammalian cells repair lipid damage using the phospholipid peroxidase glutathione peroxidase 4 (GPX4), inhibition of which causes ferroptosis, a recently described form of cell death\(^1^6\),\(^1^7\). Squalene is a lipophilic metabolite that can accumulate in cellular membranes and lipid droplets (Fig. 2h, Extended Data Fig. 2b). We therefore investigated whether squalene may be protective of lipid peroxidation and ferroptotic cell death. Blocking squalene accumulation by genetic loss of FDFT1 (Fig. 4d, Extended Data Fig. 6d–g)
small molecule inhibitors (Extended Data Fig. 7) sensitized SQLE-deficient cells to ferroptosis induced by GPX4 inhibitors (ML162 and RSL3). Extracellular squalene supplementation fails to provide control and SQLE-deficient cancer cell lines. h, Raman spectra of squalene and cholesterol for the indicated cell lines. SRS images were obtained at squalene channel (1,386 cm\(^{-1}\)) and at lipid channel (1,372 cm\(^{-1}\)) in the same cell. i, Genes ranked by differential expression analysis of primary ALK\(^+\) ALCL primary samples compared to ALK\(^-\) samples (left). Student’s t-test statistic of each gene was ranked as a function of its t-test rank. ALK\(^+\) and ALK\(^-\) normalized expression (rpm, reads per kilobase of million) of SQLE and ALK are indicated (right). b, c, f, g, Bars represent mean ± s.d.; i, boxes represent the median, and the first and third quartiles, and the whiskers represent the minimum and maximum of all data points. b, c, f, g, n = 3 biologically independent samples; i, r = 17 biologically independent ALK\(^-\) samples, 5 biologically independent ALK\(^+\) samples. Statistical significance was determined by two-tailed unpaired t-test.

Fig. 2 | ALK\(^+\) ALCLs are auxotrophic for cholesterol owing to a lack of SQLE expression. a, Immunoblotting for SQLE in LDL-dependent and independent cancer cell lines. Actin was used as a loading control. b, Relative fold change in cell viability of control and SQLE- or HSD17B7-expressing SNU-1 cancer cells grown for five days under LPDS in the absence or presence of LDL or cholesterol. c, Mass isotopomer analysis of squalene in indicated cancer cell lines in the presence or absence of LDL after a 24-h incubation with [\(\text{U}^{-13}\text{C}\)]acetate. d, Expression levels of SQLE in 1,037 cell lines from CCLE database. Cell lines with undetectable SQLE mRNA levels and their tissue origins are indicated. e, Immunoblotting for SQLE in ALK\(^+\) ALCL and control cancer cell lines. Actin was used as the loading control. f, Relative fold change in cell viability of indicated cancer cell lines grown for five days with LPDS in the absence and presence of LDL or cholesterol. g, Squalene abundance in squa
Cancer-associated alterations in metabolic enzymes influence the levels of metabolic intermediates and may thereby affect non-metabolic cellular functions such as signalling and the epigenetic state. For example, succinate and fumarate accumulate in cancers with SDH or FH mutations and may exert their pro-tumorigenic effects by acting as epigenetic modulators. Here we find that ALK+ ALCLs lose squalene monooxygenase activity and accumulate squalene, a metabolite with antioxidant-like properties (Fig. 4f).

As activation of cellular antioxidant defence is selected for in some tumour types or under certain stresses and may protect cancer cells from ferroptosis, SQLE suppression may be an additional mechanism underlying this cancer hallmark. Future work is required to understand the role of squalene accumulation in cancer initiation and progression. In addition, our work identifies cholesterol auxotrophy of ALK+ ALCLs as a targetable metabolic liability and adds ALCLs to the small list of cancers that are selectively auxotrophic for a particular nutrient. As a block in LDLR uptake inhibits the growth of ALCL tumours in our pre-clinical PDX models, inhibitors of LDLR or approaches that decrease serum cholesterol without increasing liver cholesterol may be a potential therapeutic target.

**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/s41586-019-0945-5.

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Author contributions K.B. and J.G.-B. conceived the project and designed the experiments. J.G.-B. performed most of the experiments with help from L.B. and R.G. L.B. performed CRISPR screens. L.B. and T.B. assisted with tumour xenograft experiments. E.C.B. and B.Y. performed cell competition experiments. K.L. performed computational analysis. Y.S. and W.M. designed SRS chemical imaging and generated images. G.J. and D.F. provided primary and patient-derived tumour models and performed immunohistochemistry. S.H.C., C.L. and E.F. performed lipidomics. K.B. and J.G.-B. wrote the manuscript with edits derived from D.M.S.

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. Cell lines, compounds and constructs. Antibodies to SQLE were purchased from ProteinTech (12544-1-AP, immunoblotting) and Atlas Antibodies (HPA020762, immunohistochemistry). Beta-actin (GTX109639), ACSL4 (GTX100260) and GAPDH (GTX627408) antibodies were obtained from GeneTex; LDL receptor (ab52818), NPC1 (ab36983) and GPX4 (ab14778) antibodies from Abcam; FDT1 antibody from ProteinTech (13128-1-AP); and ALK (C26707), STAT3 (9132), phospho-STAT3 (9131) and Histone H3 (4499) antibodies from CST.

Hors eradish peroxidase (HRP)-conjugated anti-rabbit antibody was purchased from Santa Cruz; mouse IgG1 isotype control antibody from BioCell; Matrixgel from Corning; fetal bovine serum, penicillin, streptomycin, and glucose from Sigma; basic fibroblast growth factor from Invitrogen; positively charged liposomes were purchased from Avanti Polar Lipids. (4-NB) from Alfa Aesar; crizotinib, atorvastatin, 5-azacytidine and 5-Aza-2′-deoxycytidine (Deoxica) from Sigma; and Shield-1 from ChemInform Pharma.

Cell culture images were taken with a REVOLVE microscope (Echo Laboratories).

All cell lines used in this study were purchased from ATCC and DSMZ or a gift of Sabatin and Weinberg labs. The identities of all the cell lines used in this study were authenticated by single tandem repeat profiling. All the cell lines were routinely tested for mycoplasma contamination every 2 weeks. Two of the cell lines, two of them were in ICLAC as misidentified cell lines but included in our analysis for diversity owing to their oncogene status and metabolic phenotypes: U-937 is a rare histiocytic lymphoma cell line described as axotrophic for cholesterol, and NCI-H929 is a myeloma cell line with low GLUT3 expression.

All cell lines were cultured in RPMI medium (Gibco) containing 1 mM glutamine, 10% fetal bovine serum, penicillin and streptomycin. For proliferation experiments in the absence of serum lipoproteins, regular RPMI was supplemented with 10% LPDS and luminescence was read after 6 days of growth. Data are presented as relative fold change in luminescence to that of cells grown in LPDS medium supplemented with 100 µg/mL LDL. In cholesterol rescue experiments, 100 µg/mL LDL (corresponding to total 50 µg/ml of cholesterol) or 10 µg/ml free cholesterol were used, as higher free cholesterol levels impaired viability of cell lines.

Of note, in our proliferation assays, we observed that glutamine levels—which correlate with the freshness of the culture medium—affect the dose of ML162 required to reduce cell proliferation and viability.

Real-time PCR assays. RNA was isolated by a RNeasy Kit (Qiagen) according to the manufacturer’s protocol. RNA was spectrophotometrically quantified and equal amounts were used for cDNA synthesis with the Superscript II RT Kit (Invitrogen). qPCR analysis was performed on an ABI Real Time PCR System (Applied Biosystems) with the SYBR green Master Mix (Applied Biosystems). The primers used were: human SQLE forward, 5′-TTCTTGTACCAGCTTATTTGT-3′; SQLE reverse, 5′-AGGGTTAGGACAGACAAAG-3′; HSD17B7 forward, 5′-GACCTTTTTGTGTGTTTGG-3′; HSD17B7 reverse, 5′-ACGGAAGATCTAACATTGG-3′; LDLR forward, 5′-TAATCCG-3′; LDLR reverse, 5′-AAACGTGCTGCTGCTGCTGCTGCTG-3′; ACSL4 forward, 5′-AAACTGCTGGTCTTCCAGATGTC-3′; ACSL4 reverse, 5′-AAACTCCGGATTATTTGC-3′; HSD17B7 forward, 5′-GGCGGACAGAATGGGCATTGGGA-3′; HSD17B7 reverse, 5′-ACGGAAGATCTAACATTGG-3′; LDLR forward, 5′-TAATCCG-3′; LDLR reverse, 5′-AAACGTGCTGCTGCTGCTGCTGCTG-3′; NPM forward, 5′-CGTCAATCGTGCTGCTGCTGCTGCTG-3′; NPM reverse, 5′-CTGCTGCTGCTGCTGCTGCTGCTG-3′.

Dil-LDL uptake. Cells were washed twice in HBSS (Gibco) and resuspended (250,000 cells per replicate) in 0.5 ml of LPDS medium supplemented with 5 µg/ml of Dil-LDL. To measure non-specific emission, one control well was used that was incubated with a Dil-LDL control reagent for 3 days. The knockout of the target gene was verified by immunoblotting. Protein Westerns were run on 4-20% gels and transferred to nitrocellulose membranes. Membranes were blocked by adding 5% non-fat milk in TBST (1× Tris-buffered saline) for 1 h and then exposed to respective primary antibodies overnight at 4°C. Membranes were washed three times in TBST for 5 min each and then incubated with the secondary antibodies for 1 h. Membranes were washed three times in TBST for 5 min each and then incubated with the secondary antibodies for 1 h. Membranes were washed three times in TBST for 5 min each and then incubated with the secondary antibodies for 1 h. Membranes were washed three times in TBST for 5 min each and then incubated with the secondary antibodies for 1 h.

Generation of knockdown and cell line overexpression cell lines. For inducible knockdown experiments, sgRNAs targeting LDLR (5′-GCTTGGAGGATGGGGCCCTC-3′), ACSL4 (5′-GAATACAGGCAAGCCAAACCCAG-3′) or a control sgRNA (AAVS1, 5′-GGGGGCCCTAGGGGACAGAGT-3′) were cloned into lentiviral DD-Cas9 vector. This vector was transfected into HEK293T cells with lentiviral packaging vectors VSV-G and Delta-VPR using XtreMeGene transfection reagent (Roche). For transduction, indicated cells were spin-infected in 6-well tissue culture plates using 8 µg/ml of polybrene at 1.200 µg for 1 h and selected for 5 µg/ml puromycin before addition of 250 nM Shield-1 reagent for 3 days. The knockdown of the target gene was verified by immunoblotting. For generation of FDT1-knockout cells or SQLE-knockout cells, an sgRNA targeting FDT1 (5′-CCCGGAGAATGGGCATTGGGA-3′) or SQLE (5′-GGACAGTGTGCTGCTGCTGCTGCTG-3′) was cloned into lentiviral CRISPR-v2. After transduction and selection using puromycin, cells were single-cell cloned. For overexpression of SQLE, guide-resistant version of FDT1, LDLR, or its dead kinase version, retroviral vectors with indicated cDNAs were transfected with retroviral packaging plasmids Gag-pol and VSV-G into HEK293T cells. After transduction, cells were selected with blastidicin.

Metabolite profiling and isotope tracing. For lipid metabolite profiling experiments, indicated cell lines (5×10⁶ cells per replicate) were cultured in 6-well plates and treated for 24 h with ML162 (200 nM), Fen-1 (1 µM), ZA (20 µM), 4-NB (1 µM) or atorvastatin (1 µM) before collection of cells and two consecutive washes with 1 ml of cold 0.9% NaCl. To measure sulfolipid synthesis,
SNU-1 cells were seeded in 60 mm dishes at 70% confluence. After 24 h, cells were given fresh medium with [14C]sodium acetate (10 μM). Cell pellets were resuspended in 600 μl of cold LC/MS grade methanol, and non-polar metabolites extracted by consecutive addition of 300 μl LC/MS grade water followed by 400 μl LC/MS grade chloroform. After 10 min extraction by vortexing and centrifugation for 10 min at 10,000 g and 4 °C, the lower lipid-containing layer was carefully collected and dried under nitrogen. Dried lipid extracts were stored at −80 °C until LC/MS analysis.

Lipids were separated on an Ascentis Express C18 2.1 mm × 150 mm × 2.7 μm column (Sigma Aldrich) connected to a Dionex UltiMate 3000 UPLC system and a QExactive benchtop orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ionization (HESI) probe. Dried lipid extracts were reconstituted in 50 μl 65:30:5 acetonitril:isopropanol:water (v/v/v) and 5 μl of sample were injected into the LC/MS/MS, with separate injections for positive and negative ionization modes. Mobile phase A in the chromatographic method consisted of 60:40 water:acetonitrile with 10 mM ammonium formate and 0.1% formic acid, and mobile phase B consisted of 90:10 isopropanol:acetonitrile, with 10 mM ammonium formate and 0.1% formic acid. The chromatographic gradient has previously been described 31. The column oven and autosampler were held at 55 °C and 4 °C, respectively. The mass spectrometer parameters have previously been described 24. The spray voltage was set to 4.2 kV, and the heated capillary and the HESI II were held at 320 °C and 300 °C, respectively. The S-lens RF level was set to 50, and the sheath and auxiliary gas were set to 35 and 3 units, respectively. These conditions were held constant for both positive and negative ionization mode acquisitions. External mass calibration was performed every seven days using the standard mass ladder mixture.

Mass spectra were acquired in both full-scan and data-dependent MS/MS mode. For the full-scan acquisition, the resolution was set to 70,000, the AGC target was 1 × 106, the maximum injection time was 50 ms, and the scan range was m/z = 133.4−2,000. For data-dependent MS/MS, the top 10 ions in each full scan were isolated with a 1.0 Da window, fragmented with a step-wise collision energy of 20−100, and the AGC target was 2 × 105 and a maximum injection time of 100 ms. The underfill ratio was set to 0. The selection of the top 10 ions was set to isotopic exclusion, a dynamic exclusion window of 5.0 s, and an exclusion list of background ions based on a solvent blank. High-throughput identification and relative quantification of lipids was performed separately for positive and negative ionization mode data using LipidSearch software (Thermo Fisher Scientific/Mitsui Knowledge Industries) 33,34 and the default parameters for QExactive product search and alignment. After alignment, raw peak areas for all identified lipids were exported to Microsoft Excel and filtered according to the following predetermined quality control criteria: Rej (‘Reject’ parameter calculated by LipidSearch) equal to 0; FP (‘Peak Quality’ parameter calculated by LipidSearch software) greater than 0.85; CV (standard deviation of retention time deviations across triple quadrupole scans) greater than 0.4; and a signal-to-noise ratio in LipidSearch (biological sample) below 0.4; R (linear correlation across a three-point dilution series of the representative (pooled) biological sample) greater than 0.9. Typically, approximately 70% of identified lipids passed all four quality control criteria. Raw peak areas of the filtered lipids were added together to create a total lipid signal for each sample, and individual lipid peak areas were normalized to this total signal as a control for lipid extraction efficiency and, as well as sample loading. Of note, in the total lipid quantification of FDTI knockout Karpas299 cells compared to rescued control, we observed an enrichment of membrane phospholipids containing saturated fatty acids (Extended Data Fig. 7c), probably due to the fact that these species are more resistant to oxidative stress and may replace or be enriched in membrane lipids upon oxidative stress.

To measure cholesterol, lanosterol, squalene and CoQ10, a XCalibur QuanBrowser 2.2 (Thermo Fisher Scientific) was used a 5-ppm mass tolerance and referenced an in-house library of chemical standards. Total lipid signal calculated with LipidSearch was used to normalize raw peak areas to generate relative abundance.

**Immunoblotting.** Cell pellets were washed twice with ice-cold PBS before lysis in lysis buffer (10 mM Tris-Cl pH 7.5, 150 NaCl, 1 mM EDTA, 1% Triton X-100, 2%, SDS, CHAPS 0.1%) supplemented with protease inhibitors and PhosSTOP (Roche). Each cell lysate was sonicated and, after centrifugation for 10 min at 4 °C and 20,000 g, supernatants were collected and their protein concentration determined by a Pierce BCA Protein Assay Kit (Thermo Scientific) with bovine serum albumin as a protein standard. Samples were resolved on 8% or 12% SDS–PAGE gels and analysed by immunoblotting as previously described 24, 25.

**Immunohistochemistry staining.** Immunohistochemistry staining was performed as previously described 24, 25. Briefly, 5-μm thick paraffin sections were incubated with primary antibody for 1 h at room temperature. After washing with PBS, sections were incubated with biotinylated anti-mouse antibody and then with biotinylated converter control DNA. Following primary validation, samples were biotinylated using the EZ DNA Methylation-Lightning Kit (Zymo D5030) according to the manufacturer’s instructions. Multiplex amplification of all samples with specific primer pairs and the Fluidigm Access Array System was performed according to the manufacturer’s instructions. The resulting amplicons were pooled for collection and subsequent barcoding according to the Fluidigm instrument’s guidelines. After barcoding, samples were purified by a ZR-96 DNA Clean & Concentrator (Zymo D4023) and then prepared for massively parallel sequencing using a MiSeq V2 300 bp Reagent Kit and paired-end sequencing protocol according to the manufacturer’s guidelines.

**Crispr-based screen.** The highly focused metabolism sgRNA library was designed as previously described by including representation of key genes of every mammalian metabolic pathway (Supplementary Table 3). Oligonucleotides for sgRNAs were synthesized by Integrated DNA Technologies and annealed before they were introduced into anti-CRISPR-v2 vector using a T4 DNA ligase kit (NEB). In brief, oligonucleotides were mixed in equal amounts and the mixed population was cultured under indicated conditions for 15 days. Cells were then collected for genomic DNA and processed for Illumina deep sequencing. Barcode abundance was determined in the starting population and after the growth.

**Ldlr monoclonal antibody purification.** Hydromed cell lines producing a monoclonal antibody against LDLR have previously been described 26. These cell lines were cultured in RPMI supplemented with 20% FBS and 2 mM pyruvate. LDLR antibody was then purified using a Protein G resin (GenScript) and Protein G Sepharose 4 Fast Flow (GE Healthcare) following the manufacturer’s instructions. C11-BODIPY lipid peroxidation. Cells were plated (250,000 cells per well) in six-well plates and treated with MIL162 for 18 h before two washes with HBSS and incubation of cells in 500 μl of HBSS containing BODIPY 581/591 C11 (1 μM). After 15 min incubation at 37 °C, cells were washed twice on ice-cold HBSS and resuspended in 0.5 ml of HBSS containing 50 ng ml−1 DAPI and filtered into FACs tubes with cell-strainer cap (Falcon). Flow cytometry data were collected on a BD LSR II Flow Cytometer (BD Biosciences) by using an excitation wavelength of 488 nm and the FL1 collection channel. FlowJo v10 software was used for data analysis. Live cells were selected from the starting cell population on a DAPI/FSC-A plot. Then, single cells were selected using a FSC-A/FSC-H plot from the population of live singlet cells. Data represents populations of live singlet cells.

**Raman Spectroscopy and Srs Microscopy.** Spontaneous Raman spectra were acquired by a confocal Raman microspectroscopy (Xplora; HORIBA Jobin Yvon) equipped with a 532-nm (40-mW) laser and a 100× objective
Chemo-immunotherapy. Patient IL69 corresponds to a 20-year-old male patient diagnosed with ALK⁺ ALCL and received two cycles of CHOP followed by two additional three cycles of BV-CHOP. On progressive disease, the patient was treated with Crizotinib but he died with active systemic disease and CNS involvement after a month. IL79 was a 65-year-old male patient with a relapsed ALK⁺ ALCL treated with BV-CHOP versus CHOP, who also died with progressive disease after four months of therapy (Supplementary Table 5).

Mini-compilation assay. Five control sgRNAs targeting intergenic regions, four sgRNAs targeting LDLR, and five sgRNAs targeting FDEFT were cloned into linearized lentiCRISPR-v2 vector and transfected in NEB competent E. coli. Each plasmid was then pooled at equal concentrations and used for lentivirus production as previously described. DEL cell line was infected and selected with puromycin for three days before being in vitro cultured or injected subcutaneously in NGS mice. Similarly, collagenase-digested ALCL PDX cells were transduced with the same lentiviral particles and subsequently injected subcutaneously in immunodeficient mice 24 h after infection. No antibiotic selection was performed as these cells cannot grow under standard culture conditions. Tumours were collected after 2–4 weeks of growth. An initial pool of each sample was taken for normalization. After 14–21 days guide DNAs were isolated and amplified by PCR. PCR amplicons were then sequenced on a NextSeq 500 (Illumina). Guide scores were calculated as median log; fold change in the abundance between the initial and final population of that sgRNA similar to standard CRISPR screens.

Mouse studies. All animal studies and procedures were conducted according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the Rockefeller University. All mice were maintained on a standard light–dark cycle with food and water ad libitum. For in vivo LDR experiments, xenograft tumours were initiated by injecting 1 × 10⁶ cells per 100 μl PBS of ALCL or control cell lines subcutaneously. For ALCL cells coming from PDXs, injections contained 1 × 10⁶ cells per 100 ul 30% Matrigel. After injections in the left and right flanks of male and female 6–14-week-old NOD scid gamma (NSG) mice (Taconic), tumours were grown for 2–4 weeks. For patient-derived tumour xenograft models, NSG B2m and NSG mice were handled according to Well Cornell Medical Institute's Institutional Animal Care and Use Committee-approved protocol 2014-0024. All operatively resected tumours were collected after written patient consent and in accordance with the institutional review board approved protocols of Well Cornell Medical Institute (2014-0024, 02010002595R012, 1401015560A002 and 0107004999).

For tumour experiments with FDEFT1-knockout and SQUEL-expressing Karpa299 cell lines, 5–10 × 10⁶ cells per 100 μl PBS of each cell line were injected subcutaneously into NGS mice and grown for 28 days. Injection of higher number of cells resulted in a loss of significance in tumour size difference, a result that is consistent with previously published work²⁶.

In no cases did xenograft tumour size surpassed the limit permitted by our protocol. All tumour studies were randomized and injections were performed by blinded investigators.

Statistics and reproducibility. GraphPad PRISM 7 and Microsoft Excel 15.21.1 software were used for statistical analysis. All experiments were performed at least two times with similar results. Both technical and biological replicates were reliably reproduced.

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

Data availability

Source data for barcoding experiment in Fig. 1 are provided as Supplementary Table 1. Gene correlation comparing RNA-sequencing data of ALK⁻ and ALK⁺ patients (Fig. 2i) is included in Supplementary Table 2. Gene scores of CRISPR screens in Fig. 3 and Extended Data Fig. 4 are provided as Supplementary Table 4. Clinical data of the PDXs used in Fig. 3f and Extended Data Fig. 6c are provided as Supplementary Table 5. Source Data for Figs. 1–4 and Extended Data Figs. 1–10 are available with the online version of the paper. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Extended Data Fig. 1 | Extracellular cholesterol dependence of cancer cell lines. a, Representative bright-field micrographs of U-937 cells cultured with the indicated concentrations of cholesterol and LDL. b, Relative fold change in cell number of indicated cell lines cultured for five days with LPDS in the presence or absence of free cholesterol relative to LDL-replete serum. c, Relative fold change in cell number of indicated cell lines grown for five days under LPDS with or without free cholesterol (1, 5 or 10 μg ml⁻¹) relative to LDL-replete serum. d, Fold change in cell viability of cholesterol auxotrophic cancer cell lines grown for five days with LPDS in the presence or absence of cholesterol or oleic acid (OA), relative to LPDS supplemented with free cholesterol. e, Reported alterations in copy number or driver mutations in oncogenic EGFR/Ras and PI3K pathways of cancer cell lines used in the DNA barcode-based competition assay. f, Heat map showing mRNA expression levels of cholesterol metabolism genes in LDL-dependent and -independent cancer cell lines. Colour bar indicates scale (log2 transformed). g, SQLE and HSD17B7 mRNA levels in indicated cell lines relative to cholesterol prototroph cell line NCI-H524. mRNA levels were measured using a real-time PCR assay. RPL0 is used as a control. h, Immunoblotting of SQLE in SNU-1 cell lines transduced with a control vector or an SQLE cDNA. Actin is included as a loading control. i, Schematic depicting squalene synthesis from acetate. In b–d and g, bars represent mean ± s.d. For b–d, n = 3 biologically independent samples. For g, n = 2 biologically independent samples. Statistical significance was measured by two-tailed unpaired t-test.
Extended Data Fig. 2 | Promoter hypermethylation of the SQLE gene and accumulation of squalene in lipid droplets of ALK+ ALCLs.

**a**, Raman spectra of squalene (blue dashed), cholesterol (red dashed) and lipid droplets in Karpas299 parental cell (blue solid), and lipid droplets in Karpas299 cell expressing SQLE cDNA (red solid). Lipid droplets were identified in bright field and targeted in the confocal Raman microspectrometer. Arrows indicate squalene-specific Raman peak. **b**, Representative bright-field image, SRS image obtained at cell lipid background (1,372 cm$^{-1}$) and fluorescence of Nile Red staining (for lipid droplets) in Karpas299 cells. **c**, Heat map showing the DNA methylation ratio for the indicated genomic region containing SQLE promoter for indicated cancer cell lines. Chromosomal position range and strand is indicated. Colour bar indicates scale. **d**, SQLE promoter methylation ratio of control (grey) and SQLE-deficient (blue) cancer cell lines. The boxes represent the median and the first and third quartiles, and the whiskers represent the minimum and maximum data points still within 1.5 of the interquartile range (n = 67 independent genomic positions per sample). **e**, Fold change in SQLE mRNA expression levels of indicated cell lines after treatment with decitabine (500 nM for 4 days) or 5-azacytidine (5-Aza, 1 μM for 6 days), relative to untreated cells (mean ± s.d., n = 3 biologically independent samples).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Lack of SQLE expression in primary ALK+ ALCLs. a, List of most-upregulated and -downregulated genes from differential expression analysis of primary ALK+ primary samples compared to ALK− samples. The student t-test statistic of each gene is calculated and used as a ranking metric (n = 17 biologically independent ALK− samples, 5 biologically independent ALK+ samples). b, Fold change in SQLE mRNA expression levels of primary ALK+ ALCLs relative to primary ALK− ALCLs, using actin as a control. The boxes represent the median and the first and third quartiles, and the whiskers represent the minimum and maximum of all data points. Statistical significance was determined by two-tailed unpaired t-test. c, Immunoblotting of SQLE and ALK in indicated PDX and cell line models. Actin was used as the loading control. PTCL, peripheral T cell lymphoma. d, Immunohistochemical staining of SQLE in ALK+ and ALK− ALCL primary tumour samples. e, Immunohistochemical staining of SQLE in Karpas299 xenograft tumours transduced with a control or SQLE cDNA. Representative images are shown. f, Immunoblotting of SQLE of indicated cell lines (top). Relative fold change in cell viability of the indicated ALK+ (Karpas299) and ALK− cell lines (TLBR-1 and ALK− PDX cell line) grown for five days under LPDS with or without free cholesterol relative to LDL-replete serum (bottom) (mean ± s.d., n = 3 biologically independent samples). g, Immunoblotting of STAT3, phospho-STAT3 and SQLE in indicated cell lines after 72-h treatment with crizotinib (200 nM). Actin was used as a loading control. h, Immunoblotting of STAT3 and phospho-STAT3 3 days after transduction of Ba/F3 with a dead kinase version of the NPM–ALK fusion (NPM-ALK DK) or with oncogenic NPM-ALK cDNA. i, SQLE mRNA levels of Ba/F3 and ALK− ALCL cell lines two or seven days after transduction with NPM-ALK DK or NPM-ALK, relative to levels in NPM–ALK dead kinase. mRNA levels were quantified with a real-time PCR assay using β-actin as a control (mean ± s.d., n = 3–4 biologically independent samples). In b, the boxes represent the median, and the first and third quartiles, and the whiskers represent the minimum and maximum of all data points. In f and h, bars represent mean ± s.d. For f and h, n = 3 biologically independent samples. Statistical significance was determined by two-tailed unpaired t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | LDLR is an essential gene for the growth of ALK^+ ALCLs. a, Squalene and lanosterol abundance of Karpas299 and DEL cell lines in the absence or presence of SQLE cDNA or after incubation for 24 h with an SQLEi (1 μM). b, Gene essentiality scores for control or SQLE-expressing DEL cell line. Pearson correlation coefficients are indicated. Red dot denotes LDLR. c, Gene essentiality scores for cholesterol-prototroph HEL cell lines in the presence or absence of an SQLEi (1 μM). Pearson correlation coefficients are indicated. Red dot denotes LDLR. d, LDLR guide scores of the indicated cell lines in the presence or absence of SQLE inhibitor. e, Immunoblots for LDLR and SQLE in control and SQLE-cDNA-expressing Karpas299 cells infected with sgAAVS1 or sgLDLR virus in the presence or absence of Shield-1 (250 nM; left). Relative fold change in cell viability of indicated cancer cell lines grown in the absence and presence of Shield-1 for 5 days (right). f, Immunoblotting of LDLR in ALK^+ ALCL lines transduced with an inducible sgLDLR vector in the presence or absence of Shield-1 (250 nM). Actin is used as a loading control. g, Relative fold change in cell viability of control or SQLE-expressing DEL cell lines transduced with sgAAVS1 or sgLDLR after five days of growth. h, Gene essentiality scores for untreated or cholesterol-supplemented Karpas299 cell line. Red dot denotes LDLR. i, LDLR guide scores in Karpas299 cell lines expressing a control vector or SQLE cDNA in the presence or absence of cholesterol supplementation. j, Coomassie blue staining of control IgG and LDLR monoclonal antibodies used in proliferation assays. k, Relative fold change in cell viability of indicated cancer cell lines grown for five days in the presence of the indicated amounts of IgG or a monoclonal antibody against LDLR. l, Relative fold change in cell viability of DEL cell lines transduced with a control vector or an SQLE cDNA grown for five days in the presence of the indicated amounts of IgG or an anti-LDLR monoclonal antibody compared to cells grown in the absence of both. In a, d, e, g, i, k and l bars represent mean ± s.d. For a, e, g, i, and k, n = 3 biologically independent samples. For d and i, n = 5 independent LDLR-targeting sgRNAs. Statistical significance was determined by two-tailed unpaired t-test.
Extended Data Fig. 5 | Upregulation of the LDL-cholesterol uptake pathway in ALK+ ALCLs. a, Dil-LDL uptake in the indicated cell lines. Results were normalized to protein levels (mean ± s.d., n = 2 biologically independent samples). b, mRNA expression levels of LDLR (log) in cell lines from CCLE database compared to that of ALK+ ALCL lines (mean ± s.d., n = 1,010 independent cell lines for CCLE collection, 5 independent cell lines for ALCL). c, Expression levels of Niemann-Pick C1 protein (NPC1) mRNA (log) in cell lines from CCLE database compared to that of ALK+ ALCL lines (mean ± s.d., n = 1,010 independent cell lines for CCLE collection, 5 independent cell lines for ALCL). d, Immunoblot of LDLR in the indicated primary PDXs (top). Immunoblotting of NPC1 in control and ALK+ ALCL cell lines (bottom). Actin is included as a loading control. PTCL, peripheral T cell lymphoma. e, Immunoblotting of SREBP-2 (non-cleaved and cleaved forms) in cytoplasmic and nuclear fractions of indicated cell lines expressing a vector or an SQLE cDNA. The cells were incubated for 24 h in medium containing either FBS (−) or LPDS (+). GAPDH and histone H3 were used as cytoplasmic and nuclear loading controls respectively.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Squalene accumulation leads to resistance of SQLE-null cells to ferroptosis inducers. a, Immunoblots of FDFT1 and SQLE in the indicated Karpas299 cell lines. Actin was used as the loading control. b, SRS imaging of squalene for indicated Karpas299 cells. Grey image shows cellular background (1,372 cm\(^{-1}\)), squalene image (pseudocoloured yellow hot, 1,386 cm\(^{-1}\); left), SRS spectra integrating intensity from lipid droplet with Raman peak of squalene (1,386 cm\(^{-1}\); right) (mean ± s.d., \(n = 3\) biologically independent samples). Error bar represent standard deviation from multiple lipid droplets in at least three cells. c, sgRNA competition assay using a pool of five control (sgControl) and five FDFT1-targeting (sgFDFT1) sgRNAs in indicated PDXs. Transduced cells were injected subcutaneously to NSG mice to generate tumours. Subsequent to four weeks of growth, genomic DNA was collected to measure sgRNA abundance by deep sequencing. Average guide scores of tumours were calculated and graphed. d, Relative fold change in cell viability of indicated Karpas299 lines treated with or without ML162 (20 nM, top) or RSL3 (30 nM, bottom) in the presence or absence of an SQLE inhibitor (1 \(\mu\)M) for 5 days. e, Fold change in cell viability relative to untreated cells of indicated Karpas299 lines treated with or without ML162 (120 nM) for 2 days (top). Representative bright-field micrographs of indicated Karpas299 cells after two days of indicated treatments (bottom). f, Immunoblotting of FDFT1 in the indicated DEL and SUP-M2 cell lines. Actin is used as a loading control (left). Relative fold change in cell viability of control, FDFT1-null and rescued DEL and SUP-M2 cell lines in the presence and absence of ML162 (20 nM) after 5 days. g, Immunoblotting of FDFT1 in the indicated HEC1B and SNU-1 cell lines. Actin is used as a loading control (top). Squalene abundance of the indicated cell lines (middle). Relative fold change in cell viability of control and FDFT1-null HEC1B and SNU-1 cell lines in the presence and absence of ML162 (200 nM for HEC1B lines, 1 \(\mu\)M for SNU-1 cell lines) and grown for 5 days. In c–g, bars represent mean ± s.d. For c, \(n = 5\) independent sgRNAs targeting a control region or LDLR gene. For d–g, \(n = 3\) biologically independent samples. Statistical significance was determined by two-tailed unpaired \(t\)-test.
Extended Data Fig. 7 | Blocking squalene accumulation sensitizes ALCLs to a GPX4 inhibitor (ML162) and erastin. **a**, Mevalonate pathway in mammalian cells and fates of the side reactions. Reactions catalysed by HMGCR, COQ2, FDFT1 and SQLE, and chemical inhibitors of these enzymes, are indicated. **b**, Relative abundance of squalene and coenzyme Q10 in Karpas299 treated for 24 h with atorvastatin (1 μM), 4-nitrobenzoate (4-NB, 1 mM) or zaragozic acid (ZA, 20 μM) to untreated. **c**, Relative fold change in cell viability of Karpas299 cells treated with erastin (1 μM), atorvastatin (1 μM), 4-nitrobenzoate (4-NB, 1 mM), zaragozic acid (ZA, 20 μM) or a combination of two of them after 5 days to untreated. **d**, Relative fold change in cell viability compared to untreated cells of Karpas299 cells treated with ML162 (25 nM), atorvastatin (1 μM), 4-nitrobenzoate (4-NB, 1 mM), zaragozic acid (ZA, 20 μM) or a combination of 2 of them after 5 days. In **b–d** bars represent mean ± s.d. For **b–d**, n = 3 biologically independent samples.
Extended Data Fig. 8 | Loss of SQLE decreases sensitivity of cancer cell lines to ferroptosis inducers. a, Immunoblotting of SQLE in the indicated cell lines transduced with a vector or sgSQLE. Actin is used as a loading control. b, Squalene abundance in the indicated cell lines. c, Relative fold change in cell viability of control and sgSQLE-expressing cell lines in the presence and absence of ML162 (500 nM for Jurkat lines, 200 nM for RPMI 8226 and SU-DHL-8 cell lines) grown for 5 days. d, Relative fold change in cell viability of Karpas299 parental cells supplemented with the indicated concentrations of exogenous squalene to untreated cells. e, Relative fold change in cell viability of Karpas299 parental or FDFT1 null cells expressing a vector, SQLE cDNA or FDFT1 cDNA treated with or without ML162, squalene or both, to untreated cells. In b–e bars represent mean ± s.d. For b–e, n = 3 biologically independent samples. Statistical significance was determined by two-tailed unpaired t-test.
Extended Data Fig. 9 | Inhibition of PUFA synthesis prevents ferroptotic cell death in ALCLs. **a**, Immunoblotting of ACSL4 in the indicated Karpas299 cells. Actin is used as a loading control (top). Relative fold change in cell viability of indicated Karpas299 cell lines in the presence or absence of ML162 (20 nM) and Fer-1 (1 μM) for 5 days (bottom) (mean ± s.d., n = 3 biologically independent samples). Statistics, two-tailed unpaired t-test. **b**, Correlation of mRNA levels of ACSL4 with SQLE (left) and ALK (right) in primary ALCLs dataset (n = 22 biologically independent samples). **c**, Correlation of mRNA levels of ACSL4 with SQLE in CCLE dataset (n = 935 independent cell lines). **d**, Lipid peroxidation assessed by flow cytometry measuring C11-BODIPY fluorescence of indicated Karpas299 cell lines after an 18-h treatment with ML162 (200 nM). Representative data from one of three experiments are shown. **e**, Lipid peroxidation assessed by flow cytometry measuring C11-BODIPY fluorescence of indicated Karpas299 cell lines after an 18-h treatment in the presence and absence of ML162 (200 nM) and Fer-1 (1 μM).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Squalene accumulation rewires membrane phospholipid composition. a, Unbiased lipidomic analysis of Karpas299 FDFT1-null cell line relative to its rescued isogenic counterpart expressing FDFT1 cDNA. Fold change (log2) in metabolite abundance was graphed and membrane phospholipids containing saturated and polyunsaturated fatty acids are indicated. b, Heat map showing fold changes (log2) in indicated phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) of Karpas299 cells cultured for 24 h in the absence or presence of ZA (zaragozic acid, 20 μM) and ML162 (200 nM) relative to untreated cells. Triplicates of each condition are shown. Colour bar indicates log2 change in abundance. c, Heat map showing fold changes (log2) in indicated phosphatidylethanolamines (PEs) of indicated Karpas299 cell lines cultured for 24 h with Fer-1 (1 μM) and ML162 (200 nM). Triplicates of each condition are shown. Colour bar indicates log2 change in abundance. d, Relative fold change in cell viability of HEL, KMS-26 and Jurkat cell lines expressing vector or an sgRNA targeting FDFT1 in the presence or absence of ML162 (20 nM) for 5 days. e, Immunoblotting of GPX4 in indicated Karpas299 cell lines expressing a vector, SQLE cDNA, FDFT1 cDNA or an sgRNA targeting FDFT1. Actin is used as a loading control. f, Coenzyme Q10 abundance of indicated Karpas299 cell lines relative to parental cells expressing a control vector. In d and f, bars represent mean ± s.d. For d and f, n = 3 biologically independent samples. Statistical significance was determined by two-tailed unpaired t-test.
Reporting Summary

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- A description of all covariates tested
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Our web collection on statistics for biologists may be useful.

Software and code

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Data collection

- For mass spectrometry: XCalibur v2.2 (Thermo Scientific) and Skyline.

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Source data for barcoding experiment in Fig. 1 is provided as Supplementary Table 1. Gene scores of CRISPR screens in Fig. 3 and Extended Data Fig. 4 are provided as Supplementary Table 4. Clinical data of Patient Derived Xenografts used in Fig. 3F and Extended Data Fig. 6C is provided as Supplementary Table 5. Source Data
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | All the experiments were performed using sample sizes based on standard protocols in the field. For primary tumor samples and Patient Derived Tumor Xenografts (PDTXs), sample size depended on the availability of patient samples for each cancer subset as provided by our collaborator. |
| Data exclusions | No data was excluded in any case. |
| Replication | All the in vitro experiments were replicated with similar outcome at least 3 times. Both technical and biological replicates were reliably reproduced. In vivo experiments were reproduced at least twice. |
| Randomization | Sample groups were allocated randomly. |
| Blinding | The investigator(s) were not blinded during data collection or analysis. For in vivo experiments, blinded researchers performed the injections. |

Reporting for specific materials, systems and methods

**Materials & experimental systems**

| n/a | Involved in the study |
| --- | --- |
| [ ] | Unique biological materials |
| [ ] | Antibodies |
| [ ] | Eukaryotic cell lines |
| [ ] | Palaeontology |
| [ ] | Animals and other organisms |
| [ ] | Human research participants |

**Methods**

| n/a | Involved in the study |
| --- | --- |
| [ ] | ChIP-seq |
| [ ] | Flow cytometry |
| [ ] | MRI-based neuroimaging |

**Antibodies**

- Antibodies used
  - Antibody, catalog number and reference describing the monoclonal LDLR antibody are included in the manuscript.
  - SQLE (Protein Tech, 12544-1-AP, Lot 00003286, Immunoblotting, 1:1000)
  - SQLE (Atlas Antibodies, HPA020762, Lot R09745, Immunohistochemistry, 1:200)
  - Beta-actin (GeneTex, GTX109639, Lot. 42810, 1:1000), ACSL4 (GeneTex, GXT100260, Lot. 39568, 1:1000), GAPDH (GeneTex, GTX627408, Lot. 41323, 1:1000), LDLR (Abcam, ab52818, Lot. GR295148-7, 1:500), NPC1 (Abcam, ab36983, 1:1000)
  - GPX4 (Abcam, ab41787, Lot. GR56784-1, 1:250), FDFT1 (Protein Tech, 13128-1-AP, Lot. 00044214, 1:1000), ALK (CST, C2667, 1:1000), STAT3 (CST, 9132), Phospho-STAT3 (CST, 9131), Histone H3 (CST, 4499S, Lot. 9, 1:500).
- Secondary Antibodies:
  - HPR-conjugated Mouse Anti-Rabbit IgG (Santa Cruz, sc-2357, Lot. No. L0617, 1:5000).
  - HPR-conjugated Donkey Anti-Mouse IgG (Santa Cruz, sc-2096, Lot. No. J2315, 1:5000).

**Validation**

Each antibody used in this work was validated prior to its use following manufacturer’s instructions.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) All cell lines used in this study were purchased from ATCC and DSMZ or a gift of Sabatini and Weinberg labs.

Authentication Short Tandem Repeat (STR) profiling of all the cell lines used in this work was performed in collaboration with the Integrated Genomics Operations Center in Memorial Sloan Kettering Cancer Center.

Mycoplasma contamination PCR analysis confirming the absence of mycoplasma contamination was performed routinely every 6 months.

Commonly misidentified lines Two of the cell lines used in this study are reported in ICLAC as misidentified cell lines but included in our analysis for diversity due to their oncogene status and metabolic phenotypes: U-937 is a rare histiocytic lymphoma cell line described as auxotrophic for cholesterol, and NCI-H929 is a myeloma cell line with low GLUT3 expression.

Animals and other organisms

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

Laboratory animals All animal studies and procedures were conducted according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the Rockefeller University and another one at Well Cornell Medicine for the Patient Derived Tumor Xenograft model. Male and female 6-14 weeks old NOD scid gamma (NSG) mice (Taconic) were used.

Wild animals This study did not involve the use of wild animals.

Field-collected samples This study did not involve the use of field-collected samples.

Human research participants

Policy information about studies involving human research participants

Population characteristics We provided a supplementary table (Supplementary Table 5) regarding patient information. Briefly, samples IL69 and IL79 (ALK+ PDX1 and ALK+ PDX3 in Fig. 3h) correspond to patients who experience a refractory clinical course unresponsive to standard chemo-immunotherapy. IL69 correspond to a 20-year-old male diagnosed with ALK+ ALC1 of the right shoulder, who received 2 cycle of CHOP followed by two additional 3 cycles of BV-CHP. On progressive disease, the patient was treated with Crizotinib but he died with active systemic disease and CNS involvement after a month. IL79 was a 65-year-old male with a relapsed ALK+ ALC1 treated with BV-CHOP vs CHOP, who also died with progressive disease after 4 month of therapy (Supplementary Table 5). Fresh and/or viable cryopreserved samples from primary ALC1 were obtained at the time of diagnosis, before treatment, or at relapse after chemotherapy. Formalin Fixed Paraffin Embedded tissue samples were obtained from multiple institutions and the diagnoses were rendered according to the WHO classification. Review of samples were performed in blind by two experienced pathologists to determine the percentage of lymphoma cells, based on H&E and immunohistochemistry (IHC) analyses on a semi-automated stained. Informed consents were obtained following the recommendations of local ethical committees.

Recruitment See above.

Flow Cytometry

Plots

Confirm that:

☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☑ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☑ All plots are contour plots with outliers or pseudocolor plots.
☑ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation Indicated cancer cell lines were plated (250,000 cells/well) in 6-well plates and treated with ML162 for 18 hrs prior to two washes with HBSS and incubation of cells in 500 uL of HBSS containing BODIPY 581/591 C11 (1 uM). After 15 min incubation at 37 °C, cells were washed twice on ice-cold HBSS and resuspended in 0.5 mL of HBSS containing 50ng/ml DAPI and filtered into FACS tubes with cell-strainer cap.

Instrument BD LSR II Flow Cytometer (BD Biosciences)

Software FlowJo v.10
Cell population abundance | N/A

Gating strategy
Live cells were selected from the starting cell population on a DAPI/FSC-A plot. Then, single cells were selected using a FSC-A/FSC-H plot from the population of live cells. Data displayed represents populations of live, singlet cells.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.