Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity

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Most tumours have an aberrantly activated lipid metabolism1–7 that enables them to synthesize, elongate and desaturate fatty acids to support proliferation. However, only particular subsets of cancer cells are sensitive to approaches that target fatty acid metabolism and, in particular, fatty acid desaturation8. This suggests that many cancer cells contain an unexplored plasticity in their fatty acid metabolism. Here we show that some cancer cells can exploit an alternative fatty acid desaturation pathway. We identify various cancer cell lines, mouse hepatocellular carcinomas, and primary human liver and lung carcinomas that desaturate palmitate to the unusual fatty acid sapienate to support membrane biosynthesis during proliferation. Accordingly, we found that sapienate biosynthesis enables cancer cells to bypass the known fatty acid desaturation pathway that is dependent on stearoyl-CoA desaturase. Thus, only by targeting both desaturation pathways is the in vitro and in vivo proliferation of cancer cells that synthesize sapienate impaired. Our discovery explains metabolic plasticity in fatty acid desaturation and constitutes an unexplored metabolic rewiring in cancers.

Cancer cells display differential usage of stearoyl-CoA desaturase (SCD)-dependent fatty acid desaturation 2 (Extended Data Fig. 1a). To illuminate this plasticity, we treated liver (HUH7), lung (A549 and H460), prostate (DU145) and breast (MDA-MB-468 and T47D) cancer cell lines with the SCD inhibitor Merck Frosst compound 3j4 in conditions of low extracellular fatty acid availability. We observed that these different cell lines exhibited a broad sensitivity profile toward SCD inhibition (Fig. 1a). On the basis of this proliferation response, we classified these cancer cells as SCD-dependent (inhibition of proliferation, or cell death), partially SCD-dependent (less than 50% proliferation) or SCD-independent (more than 50% proliferation). Next, we excluded the possibility that the differential dependency of cancer cells on SCD activity was a result of their individual growth rate, fatty acid synthesis rate or the degree to which Merck Frosst compound 3j inhibited SCD (Extended Data Fig. 1b–g). We concluded on the basis of these results that some cancer cells are SCD-independent, which cannot be explained by their known fatty acid metabolism. Consequently, we hypothesized that SCD-independent and partially SCD-dependent cancer cells exploit an alternative desaturation pathway. We reasoned that the presence of such an alternative pathway must result in the synthesis of unusual monounsaturated fatty acids, and therefore measured saturated and monounsaturated C12 to C18 fatty acids. In general, the different cancer cells presented a wide range of total fatty acid abundance, which did not correlate with SCD independence (Extended Data Fig. 1h, Supplementary Table 1a, b). Yet, we discovered an elevated abundance of the unusual fatty acid sapienate (cis-6-C16:1) in SCD-independent and partially SCD–dependent cancer cells that increased upon SCD inhibition (Fig. 1b, Extended Data Fig. 2a, Supplementary Table 1a, b).

Sapienate is a major component of human sebum and is to date considered a specific marker of sebocyte metabolism in the sebaceous glands3–6. Because sebocytes produce sapienate from palmitate, we determined the desaturation activity from palmitate to sapienate by assessing the sapienate-to-palmitate ratio and sapienate biosynthesis. Both measures increased upon SCD inhibition in the cell lines we tested, with the exception of SCD-dependent T47D cells (Extended Data Fig. 2b, c). Moreover, the sapienate-to-palmitate ratio correlated with SCD independence, and was higher in SCD-independent (HUH7) and partially SCD–dependent (DU145) cancer cells compared to non-transformed cells of the same tissue origin (Fig. 1c, Extended Data Fig. 2d). Subsequently, we discovered the desaturation activity of palmitate to sapienate in the HUH7 tumour xenografts upon SCD inhibition. Consistent with our in vitro data, we found that SCD inhibition did not significantly alter final tumour weight, but did increase the desaturation activity to sapienate (Fig. 1d, Extended Data Fig. 2e). Accordingly, we observed that diethylnitosamine- and genetically induced mouse hepatocellular carcinoma (HCC) exhibited a significantly elevated desaturation activity to sapienate, as compared to normal liver (Fig. 1e, f). These data collectively show that cancer cells, and in particular HCC, can produce sapienate both in vitro and in vivo.

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In sebocytes, sapienate is produced by fatty acid desaturase 2 (FADS2)\(^2\) (Extended Data Fig. 1a). Therefore, we investigated whether cancer cells exploit FADS2 to synthesize sapienate. We found that FADS2 gene expression was increased in SCD-independent and partially SCD-dependent cancer cells, compared to SCD-dependent cells, and in liver and prostate cancer cells upon SCD inhibition (Extended Data Fig. 2f, g). Consistently, FADS2 protein expression correlated with SCD independence and desaturation activity to sapienate in cancer cells (Fig. 1g, h). Moreover, FADS2 protein and gene expression was elevated in HUH7 and DU145 cancer cells compared to corresponding non-transformed cells (Extended Data Fig. 2h). Similarly, FADS2 gene expression was increased in matched pairs of cancer versus adjacent non-cancerous tissue of HCC (3 out of 4) and non-small-cell lung cancer (8 out of 10) from human patients (Fig. 1i, j). These data suggest an involvement of FADS2 in sapienate biosynthesis. Accordingly, FADS2 silencing resulted in a decreased desaturation activity to sapienate in vitro and in vivo (Fig. 1k, l, Extended Data Fig. 2i). These findings demonstrate that some cancer cells exploit FADS2 to produce sapienate.

Next, we investigated whether sapienate biosynthesis causes SCD independence. Sapienate supplementation or FADS2 overexpression in SCD-dependent MDA-MB-468 cells restored proliferation upon SCD inhibition and thus resulted in SCD independence (Fig. 2a, b, Extended Data Fig. 3a). Moreover, FADS2 silencing combined with SCD inhibition caused the inhibition of proliferation in HUH7 cells and cell death in A549 cells (Fig. 2c, d), whereas sole FADS2 knockdown seemed to increase proliferation in HUH7 cells. These findings indicate that some cancer cells might rely on the metabolic plasticity provided through simultaneous SCD and FADS2 desaturation activities at the expense of maximized proliferation—a phenomenon that has previously been described\(^1\). Subsequently, we assessed dual inhibition of SCD- and FADS2-dependent desaturation in HUH7 orthotopic liver xenografts. We found that only dual inhibition of SCD and FADS2 resulted in a significantly smaller tumour area compared to control tumours (Fig. 2e, f). Unlike the in vitro results, no full inhibition of tumour growth was achieved in vivo; this was probably due to the lower in vivo knockdown efficiency and a partial compensation through extra-cellular sapienate uptake (Extended Data Fig. 3b–d). An involvement of linoleate—a known substrate of FADS2 in polydesaturation—in the observed SCD independence was excluded (Extended Data Fig. 3e–h). Taken together, these data demonstrate that dual activity of SCD- and FADS2-dependent desaturation can provide metabolic plasticity that supports proliferation, which can be impaired in vitro and in vivo by the combined inhibition of both pathways.

An important fate of fatty acids is membrane synthesis, for which fatty acids are often elongated. Accordingly, we observed carbons of sapienate in its elongation product cis-8-octadecenoate (cis-8–C18:1; Extended Data Fig. 4). Consistent with sapienate elongation, the abundance of cis-8-octadecenoate was higher in SCD-independent cells than in SCD-dependent cells, increased in HUH7 and A549 cells upon sapienate supplementation or SCD inhibition, and decreased upon FADS2 silencing (Fig. 3a, b, Extended Data Fig. 5a–c, Supplementary Table 1c, d). Consistently, cis-8-octadecenoate supplementation rescued the proliferation of SCD-dependent MDA-MB-468 cells and FADS2 knockdown cells (HUH7 and A549) upon SCD inhibition (Extended Data Fig. 4).
Fig. 2 | Sapienate synthesis via FADS2 causes independence from the known SCD-catalysed fatty acid desaturation. **a, b,** Relative proliferation of MDA-MB-468 control (with or without sapienate) and FADS2 overexpression cells upon treatment with 0.5 nM Merck Frosst compound 3j, normalized to control (a, n = 9; b, control n = 10, overexpression n = 12). Two-way ANOVA with Tukey’s multiple comparisons. **c, d,** Relative proliferation of HUH7 and A549 cells (with or without sapienate) upon FADS2 knockdown, with or without 2 nM Merck Frosst compound 3j, normalized to control (c, control n = 9; shFADS2-1 n = 6; shFADS2-2 n = 6). Two-way ANOVA with Tukey’s multiple comparisons (within different cell lines); one-way ANOVA with Dunnett’s multiple comparisons (across different cell lines). Only pairwise comparisons are depicted. **e, f,** Representative images of haematoxylin and eosin staining, and relative area of resected tumour.

Data Fig. 5d–f). We then determined whether sapienate is used for membrane synthesis. FADS2 silencing altered the overall composition of membrane-bound phospholipids, decreased the fraction of phospholipids built from sapienate and increased phospholipids built from the SCD product palmitoleate in HUH7 and A549 cells (Fig. 3c, Extended Data Fig. 6a–e; Supplementary Table 1e). The opposite change occurred upon SCD inhibition (Fig. 3d, e). Functionally, these changes in membrane composition resulted in a trend towards decreased membrane fluidity and increased resistance to lipid peroxidation in FADS2 knockdown cells (Extended Data Fig. 6f, g). Thus, these data show that some cancer cell lines elongate sapienate and use it for membrane biosynthesis.

Finally, we asked whether sapienate metabolism also occurs in primary human cancers. We measured the ratio of sapienate to palmitate in cancer and normal lung and liver tissue, as well as in blood plasma from human subjects. Blood plasma was obtained from healthy volunteers or patients with cancer, and normal lung and liver was obtained from adjacent non-cancerous tissue from patients with cancer and non-transplanted donor organs, respectively. In addition, we determined the palmitoleate-to-palmitate ratio in the same tissues and blood plasma samples as a readout of the SCD-dependent fatty acid desaturation pathway. We found that in cancer tissue, but not in normal tissue, the sapienate-to-palmitate ratio was significantly increased compared to the ratio in blood plasma (Fig. 4a, b). Accordingly, the sapienate-to-palmitate ratio was higher in cancer tissue than in normal tissue (Fig. 4a, b). The increase in the sapienate-to-palmitate ratio was more pronounced than the corresponding change in the palmitoleate-to-palmitate ratio when comparing cancer and normal tissue (Fig. 4a, b). This suggests a specific increase in sapienate biosynthesis in these cancers, rather than a general increase in the synthesis of monounsaturated fatty acids. Taken together, these data provide evidence that sapienate metabolism occurs in vivo in primary lung and liver carcinomas from human patients.

The well-characterized SCD-dependent fatty acid desaturation pathway has previously been considered to be the only source of de novo-generated monounsaturated fatty acids in cancer cells. Here we show that cancer cells can rewire their fatty acid metabolism and desaturate palmitate to the unusual fatty acid sapienate (Fig. 4c). Particularly,
we find evidence for sapienate metabolism in human lung and liver carcinoma. This finding can explain metabolic plasticity, and demonstrates heterogeneity in the fatty acid desaturation metabolism of cancer cells. Although we find that sapienate and cis-8-octadecenoate can support membrane synthesis, it is tempting to speculate that sapienate and its elongation products affect the known fatty acid and lipid signalling networks of cancer cells. Consequently, this could provide cancer cells with a hitherto unexplored possibility to deregulate signalling networks, opening new opportunities to understand and target them. In conclusion, our discovery increases the current understanding of fatty acid metabolism in cancers and suggests sapienate biosynthesis as an alternative source of monounsaturated fatty acids.

Online content
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Fig. 4 | Evidence for sapienate synthesis in primary human cancers. a, Sapienate-to-palmitate and palmitoleate-to-palmitate ratios in HCC and normal liver tissue, as well as in blood plasma from humans. Blood plasma was from healthy volunteers or patients with cancer, and normal liver was adjacent non-cancerous tissue from patients with cancer. Non-transplanted donor livers (healthy, blood plasma n = 23 and tissue n = 16; cancer, blood plasma n = 33 and tissue n = 16). Notably, blood plasma ratios from healthy volunteers are the same as in b, Sapienate-to-palmitate and palmitoleate-to-palmitate ratios in lung cancers and normal lung tissue, as well as in blood plasma from humans. Blood plasma was from healthy volunteers or patients with cancer, and normal lung was adjacent non-cancerous tissue from patients with cancer (healthy, blood plasma n = 23 and tissue n = 15; cancer, blood plasma n = 34 and tissue n = 15). Notably, blood plasma ratios from healthy volunteers are the same as in a. c, Sapienate metabolism is an alternative monodesaturation pathway. Two-way ANOVA with Tukey’s multiple comparisons. Error bars represent s.e.m. of mean from different individuals.

Author contributions
In investigation and validation, K.V., S. Christen and S.P. generated knockdown and overexpression cell lines; K.V., S. Christen, S.P., D.B., C.E.-N. and L.R.-P. quantified FADS2 gene expression; K.V., S. Christen and S.P. performed growth and labelling experiments; growth experiments were performed independently by K.V. and S. Christen; R.J.D. and K.V. produced reverse labelling experiments; K.V., S. Christen, R.S. and K.Y. performed metabolite measurements; K.V., S.P., G.R. and M.R. studied FADS2 protein data; T. Cornfield, C.C. and L.H. analysed phospholipid-bound sapienate and palmitoleate; A.T. and J.D. performed phospholipidomic analysis and assessed lipid peroxidation sensitivity; A.K. analysed membrane fluidity; K.V., D.B., R.S. and C.C.K. performed mouse experiments; K.V., M.F.O., J.V.S. and L.H. advised on lipid and fatty acid analysis. J.D. performed phospholipidomic analysis and assessed lipid peroxidation sensitivity; A.K. analysed membrane fluidity; K.V., D.B., R.S. and C.C.K. performed mouse experiments; K.V., M.F.O., B.F., R.J.D., D.C., C. Verslype and T.G.P. collected and assessed human clinical samples. T.G.P. and M.F.O. performed haematoxylin and eosin staining. In formal analysis, K.V., S. Christen and S.-M.F. interpreted all data, except data from haematoxylin and eosin staining, which were interpreted by T.G.P. and M.F.O. The following authors provided resources: M.N. and E.S. provided primary hepatocytes; R.B. and C. Verfaille advised on genetic engineering and primary hepatocytes; B.F. and R.J.D. provided human lung tissue and plasma samples; D.C., C. Verslype, S. Christen, S.G., A.S. and T.G.P. provided human liver tissue and plasma samples; N.G., J.V.S. and I.H. advised on lipid and fatty acid analysis. S.M. advised on membrane fluidity; R.D., J.F.G. and J.A.G.D. supported the development of the mass spectrometry method; A.M.-L., M.Y., S.Y.K. and L.B. provided liver tissue and plasma from genetic mouse models; and A.S., P.S., A.H., J.A.G.V., S. Christain, S.G., A.S., T. Chen, K.-K.W. provided reagents. S.-M.F. advised on experiments and analysis. This work was conceptualized by K.V., S. Christen and S.-M.F. K.V. provided visualization. S.-M.F. wrote the original draft, and S.-M.F. supervised this work, and acquired funding.

Competing interests
A.H., C.C.K., A.S., P.S., S. Christian and G.S. have competing interests as employees of Bayer AG. K.-K.W. is a founder and equity holder of G1 Therapeutics and he has Consulting/Sponsored Research Agreements with AstraZeneca, Janssen, Pfizer, Array, Novartis, Merck, Takeda, Ono, Targimmune and BMS. S.-M.F. has received research funding from Bayer AG and Merck.

Additional information
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Methods

Cell lines, cell culture and chemicals. All cell lines were confirmed to be mycoplasma-free, based on the MycoAlert Mycoplasma Detection Kit (Lonza). Human HEK293T epithelial cells, RWPE-1 prostate cells, MCF10A breast cells, A549 and H460 lung carcinoma, MDA-MB-468 and T47D breast adenocarcinoma, and DU145 prostate carcinoma cell lines were obtained from ATCC. The HUH7 liver carcinoma cell line was obtained from the Japanese Collection of Research Bioresources (JCRB) Bank. Cell lines have not been authenticated, with the exception of MDA-MB-468 cells, which were authenticated by fingerprinting. RWPE-1 cells were cultured in keratinocyte serum-free medium (K-SFM), supplemented with 0.05 mg per ml bovine pituitary extract, 5 ng per ml epidermal growth factor, 1% penicillin (50 U per ml) and 1% streptomycin (50 μg per ml) (Life Technologies). MCF10A cells were cultured in Dulbecco’s modified Eagle’s medium-F12 (DMEM-F12) (Life Technologies), supplemented with 5% horse serum (Life Technologies), 1% penicillin (50 U per ml) (Life Technologies), 1% streptomycin (50 μg per ml) (Life Technologies), 0.5 μg per ml hydrocortisone (Sigma-Aldrich), 100 ng per ml chola toxin (Sigma-Aldrich), 10 μg per ml insulin (Sigma-Aldrich), and 20 ng per ml recombinant human epidermal growth factor (PeproTech EC). Other cells were cultured in high glucose (4.5 g per l) Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 1% penicillin (50 U per ml) (Life Technologies) and 1% streptomycin (50 μg per ml) (Life Technologies). For growth and labelling experiments, low serum conditions (0.5–1% FBS) were applied. 13C6-glucose (CLM-1396 Cambridge Isootope Laboratories) was used for labelling experiments. Hydrogynin B and puromycin dihydrochloride (Life Technologies) were added to the growth medium for selection of overexpressed and knockdown cell lines, respectively. Merck Frost compound 3j was used as an SCD inhibitor, as described in the patent application WO2006/130986. The fatty acids palmitoleate (16:1), sapienate (16:1) and oleate (18:1) were purchased from Sigma-Aldrich. cis-8-octadecenoate (18:1) was purchased from Larodan. Solvents for metabolite extraction and mass spectrometry were HPLC-grade from Sigma-Aldrich.

Knockdown and overexpression experiments. FADS2 knockdown cell lines were generated using the shRNA-expressing lentiviral plKO1-puro vector with a puromycin selection cassette (Plasmid 8453; Addgene). Clone identities for shRNAs were as follows: shFADS2-1 (TRCN0000064755; sequence: CCGGCCACCCGCGCAG-AATCTCAAGAATCAGTTTGGCTGGTACTGTCCGTGTTTTTGG) and shFADS2-2 (TRCN0000064757; sequence: CCGGCCGACCTGCTGCTGCTACAGAAACTCGAGTTTCTGTAGA-CAGACAGGTGGTTTTG) and shFADS2-3 (TRCN0000064758; sequence: CCGGCCGACCTGCTGCTGCTACAGAAACTCGAGTTTCTGTAGA-CAGACAGGTGGTTTTG) and shFADS2-4 (TRCN0000064759; sequence: CCGGCCGACCTGCTGCTGCTACAGAAACTCGAGTTTCTGTAGA-CAGACAGGTGGTTTTG).

FADS2 knockdown cell lines were generated using the pLVX-IRES-Hyg vector with a hygromycin selection cassette (Clontech Laboratories). An empty plLVX-IRES-Hyg vector served as a negative control for FADS2 overexpression. Lentivirus was produced by transfection of HEK293T cells. Transduction of cells was performed overnight and the medium was replaced the next day. Polyclonal cells were selected for 1–2 weeks with puromycin (in the case of knockdown cells) or with hygromycin (in the case of overexpression), before cells were performed. All knockdown and overexpression cell lines were validated by quantitative real-time PCR (qRT–PCR) and protein analysis (Extended Data Figs. 2i, 3a). Primers for FADS2 were designed to amplify a CDNA segment in the sequence as follows: forward primer 5′-gagcagcgaagaactaag-3′ and reverse primer 5′-ggaggaggaggtcattgacc-3′. For ScDI1, the forward and reverse primers used were 5′-ctctgctacctgaggg-3′ and 5′-gagggattagggaggtcattgacc-3′, respectively. Gene transcript levels were compared to the control gene RPL19, with 5′-attgtctgctgggcttaca-3′ and 5′-attgtctgctgggcttaagcag-3′ as forward and reverse primer, respectively. Relative gene transcript levels were calculated by the 2−ΔΔCT method following amplification (Thermo Fisher Scientific) following trypsinization, and relative cell number changes were calculated as described above. All experiments were performed in triplicates. Changes in intracellular fatty acid abundance upon supplementation were confirmed by mass spectrometry (Supplementary Table 1c, d).

Primary hepatocytes of one donor were obtained from the Hepatocytes and Liver Stem Cell Bank, Cliniques Universitaires St Luc. Cells were thawed using the Corning Gentest High Viability CryoHepatocyte Recovery Kit (Corning). Cells were then plated on collagen-I-coated 6-well plates at a density of 1.4 × 105 cells per well (T47D) or 1.5 × 105 cells per well (MDA-MB-468) or 2 × 106 cells per well (T47D) in low FBS DMEM (1.5 ml per well) and grown in a humidified environment at 37 °C with 5% CO2. For HUH7, 1% FBS was confirmed to be low FBS DMEM; for all other cell lines, 0.5% FBS was used. Low FBS (0.5–1%) DMEM contains a total of 3.41–8.38% BSAC (beta-sitosterol, stigmasterol and stigmasteradiol) and 1.5–2.4% other fatty acids (Extended Data Fig. 7a). After 24 h, the medium was aspirated, cells were washed with DBPS and low FBS DMEM (4.5 g per l glucose for growth experiments or 4.5 g per l 13C6-glucose for labelling experiments, including fatty acid synthesis assessment), supplemented with 0.1% DSMO (control) or Merck Frost compound 3j (either at 0.5 nM, 1 nM or 2 nM concentration dissolved in DMSO) added to the wells (1.5 ml per well). Treatment was carried out for 72 h, during which cells were grown in a humidified environment at 37 °C with 5% CO2. For growth experiments, cells were counted before treatment (initial count) and 72 h after treatment (final count) using a Mosi Z Mini Automated Cell Counter (Orflo Technologies) or a Countess II Automated Cell Counter (Thermo Fisher Scientific) following trypsinization. Cell number change was calculated by subtracting the initial count from the final count, and was subsequently normalized to the control condition, unless stated otherwise. For labelling experiments, cells were washed with saline solution after treatment and metabolism was quenched by flash-freezing the plates in liquid nitrogen. Plates were stored at –80 °C until metabolite extraction. All experiments were performed in triplicates. Key experiments were confirmed in medium with glucose and amino acid concentrations similar to blood (BLM)12,13 in low FBS (Extended Data Fig. 7b–h).

For rescue experiments, cells were seeded and grown as described above. After 24 h, the medium was replaced by medium containing 0.1% DSMO (control) or Merck Frost compound 3j (0.5 nM, 1 nM or 2 nM), supplemented with either 1% ethanol (control) or 20 μM palmitoleate (cis-9-16:1), sapienate (cis-6-16:1), oleate (cis-9-18:1) or cis-8-octadecenoate (cis-8-18:1) dissolved in ethanol. Cells were counted before treatment and 72 h after treatment using a Moxi Z Mini Automated Cell Counter (Orflo Technologies) or a Countess II Automated Cell Counter (Thermo Fisher Scientific) following trypsinization, and relative cell number changes were calculated as described above. All experiments were performed in triplicates. Changes in intracellular fatty acid abundance upon supplementation were confirmed by mass spectrometry (Supplementary Table 1c, d).

Primary hepatocytes of one donor were obtained from the Hepatocytes and Liver Stem Cell Bank, Cliniques Universitaires St Luc. Cells were thawed using the Corning Gentest High Viability CryoHepatocyte Recovery Kit (Corning). Cells were then plated on collagen-I-coated 6-well plates at a density of 1.4 × 105 cells per well, and left to attach for 8 h in Corning Plating Medium (Corning).

Reverse labelling experiments. HUH7 and A549 cells were seeded at 1 × 106 cells per flask in T75 flasks in low FBS (1% FBS, HUH7) or 10% FBS (A549) DMEM and grown as described above. After 24 h, the medium was aspirated, cells were washed with DBPS and 1% (HUH7) or 10% (A549) dialysed FBS DMEM (4.5 g per l 13C6-glucose) was added to the cells. Cells were cultured for 1 week (medium was replaced every 3 days) to fully label all cellular metabolites. Next, cells were trypsinized, washed and seeded in the wells of a 12-well plate at 7 × 105 cells per well in low FBS DMEM containing 4.5 g per l 13C6-glucose (1% FBS for HUH7 and 0.5% FBS for A549). After 24 h, the medium was aspirated, cells were washed with DBPS and low FBS DMEM containing 4.5 g per l 13C6-glucose (1% FBS for HUH7 and 0.5% FBS for A549). At the end of the experiment, labeled cells were washed with saline solution and metabolism was quenched by flash-freezing the plates in liquid
If fully labelled cells use 13C-sapienate, a decrease in the 13C enrichment will be observed in the metabolites into which sapienate is incorporated, such as 12C-sapienate or palmitoleate, and not 13C-sapienate or palmitate. If fully labelled cells use 12C-sapienate, a decrease in the 13C enrichment will be observed into the metabolites into which sapienate is incorporated, such as palmitate to sapienate or palmitoleate. Note that 12C-sapienate is not directly incorporated into 12C-sapienate, but rather is incorporated into palmitate or sapienate via the desaturation of sapienate. The desaturation activity was measured with a GC–FID system (Trace GC Ultra, Thermo Fisher Scientific) in-line-coupled to a Q Exactive HF mass spectrometer (Thermo Fisher Scientific). The peptides were first loaded on a trapping column (made in-house, 100-μm internal diameter (i.d.) × 20 mm, 5-μm beads C18 Reprosil-HD, Dr. Maisch) and after flushing from the trapping column, peptides were separated on an analytical column in the needle (made in-house, 75-μm i.d. × 400 mm, 1.9-μm beads C18 Reprosil-HD, Dr. Maisch) using a nonlinear 150 min gradient of 2 to 5% solvent B (0.1% formic acid in water/acetonitrile, 20/80 (v/v)) at a constant flow rate of 250 nl per min and at a constant temperature of 50 °C (CoControl 3.3.05, Sonation). Following a 10-min wash reaching 99% solvent B, the column was re-equilibrated with solvent A (0.1% formic acid in water/acetonitrile, 80/20 (v/v)). The mass spectrometer was operated in data-dependent, positive ionization mode, automatically switching between mass spectra and tandem mass spectra acquisition for the 16 most abundant peaks in a given mass spectrum. The source voltage was set to 2.5 kV and the capillary temperature was 250 °C. One MS1 scan (m/z 375–1,500, AGC target 3 × 106 ions, maximum ion injection time of 60 ms), acquired at a resolution of 15,000 (at 200 m/z) was followed by up to 16 tandem mass spectrometry scans, acquired at a resolution of 15,000 (at 200 m/z) of the most intense ions fulfilling predefined selection criteria: AGC target 1 × 105 ions, maximum ion injection time of 80 ms, isolation window of 1.5 m/z, fixed first mass of 145 m/z, spectrum data type: centroid, under fill ratio 2%, intensity threshold 1.3 × 105, exclusion of unassigned and singly charged precursors, peptide mass preferred, exclude isotopes on, dynamic exclusion time of 12 s. The normalized collision energy was set to 28% and the polydimethylsiloxane background ion at 45,120,003 Da was used for internal calibration (lock mass).

Data analysis was performed by MaxQuant (version 1.6.1.0) using the Andromeda search engine with default search settings, including a false discovery rate set at 1% on both the peptide and protein level. Spectra were searched against a custom database (December 2017, containing 20,243 human protein sequences; downloaded from www.uniprot.org). The mass tolerance for precursor and fragment ions was set to 4.5 and 20 p.p.m., respectively, during the main search. Enzyme specificity was set as C-terminal to arginine and lysine (trypsin), also allowing cleavage at arginine–proline or lysine–proline bonds with a maximum of two missed cleavages. Carbamidomethylation of cysteine residues was set as a fixed modification and variable modifications were set to oxidation of methionine residues (to sulfones) and acetylation of protein N termini. Proteins were quantified by the MaxLFQ algorithm integrated in the MaxQuant software. Only proteins with at least one unique or razor peptide were retained for identification, and a minimum ratio count of two unique peptides was required for quantification. The obtained label-free quantification intensity values of FADS2 were used to quantify the protein, and to compare levels of FADS2 between different samples.

**Analysis of phospholipid-bound sapienate and palmitoleate.** HUH7 cells carrying a non-targeting shRNA or a shRNA targeting FADS2 (shFADS2-2) were seeded in T75 flasks, allowed to attach for 24 h and subsequently grown for 72 h in 1% FBS DMEM. Cells were trypsinized, washed with blood bank saline and re-suspended in TAG lysis buffer (1% IGEPA L-630 (Nonidet P-40), 50 mM Trizma hydrochloride and 150 mM sodium chloride). Lipids were extracted from cell lysate according to the Folch method27. An internal standard containing a known concentration of 1,2-diheptanoyl-sn-glycer-3-phosphocholine (17:0) was added to samples before extraction to allow the quantification of total phospholipids. Lipid fractions were separated by thin-layer chromatography and fatty acid methyl esters were prepared as previously described.29,30 Separation and detection of total phospholipid fatty acid methyl esters was achieved using a 6890N Network GC System (Agilent Technologies) with flame ionization detection. Fatty acid methyl esters were identified using a data-dependent analysis (collision energy was set to 14 eV, Exclusion was set to 5 s). The total ion abundance of each phospholipid was compared to a standard containing 31 known fatty acids and quantified in micromoles from the peak area based on their molecular mass. The micromole quantities were then totalled and each fatty acid was expressed as a percentage of this value.
molar percentage (mol%), or as micrograms of fatty acids normalized to cellular protein amount.

**Analysis of phospholipid species.** HUH7 and A549 cells carrying a non-targeting shRNA or one of two shRNAs targeting FADS2 (shFADS2-1 or shFADS2-2) were seeded in T75 flasks, allowed to attach for 24 h and subsequently grown for 72 h in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Cells were trypsinized, washed three times with cold DPBS and cell pellets were re-suspended in 0.8 mL DPBS. Lipid extraction and multiple reaction monitoring (MRM)-based phospholipid (sem)quantification analysis was performed as previously described. In brief, 0.7 mL of homogenized cells were mixed with 0.9 mL MeOH:HCl (1 M) (8:1), 0.8 mL CHCl3 and 200 μg per mL of the antioxidant 2.6-di-tert-butyl-4-methylphenol (Sigma). The organic fractions were evaporated under vacuum using a Savant SpeedVac SPD117 (Thermo Fisher Scientific) at room temperature and the remaining lipid pellet was stored at −20 °C under argon. Before mass spectrometry analysis, lipid pellets were reconstituted in running solution (CH3OH:CH3CN:NH4OH; 90:10:1.25, v/v/v). The lipid standards phosphatidylcholine (PC)25:0, PC43:6, sphingomyelin (SM)30:1, phosphatidylethanolamine (PE)25:0, PE34:6, phosphatidylinositol (PI)25:0, PI31:1, PI43:6, phosphatidylserine (PS)25:0, PS31:1 and PS37:4 (Avanti Polar Lipids) were added based on the amount of DNA of the original sample. Phospholipids were analysed by electrospore ionization tandem mass spectrometry (ESI-MS/MS) on a hybrid quadrupole linear ion trap mass spectrometer (4000 QTRAP system, AB SCIEX) equipped with a TriVersa NanoMate robotic nanosource (Advinion Biosciences) for automated sample injection and spraying as previously described. Phospholipid profiling was executed by (positive or negative) precursor ion or neutral loss scanning at a collision energy of 30 eV or 45 eV, 35 eV, 35 eV, 36 eV and 60 eV for precursor 184 (SM or PC), neutral loss 141 (PE), neutral loss 87 (PS) and precursor 241 (PI), respectively. Phospholipid quantification was performed by MRM, the transitions being based on the neutral losses or the typical product ions as described above. Typically, a 3-min period of signal averaging was used for each spectrum. The data were corrected for carbon isotope effects and chain length, and analysed using in-house-developed software (RALP). As a background, the intensities of species detected in the ‘internal standards only’ spectra were considered after being divided by the ion suppression factor of each sample. The ion suppression factor was calculated for each sample separately by dividing the intensity of the standards in the ‘internal standards only’ spectrum by the intensity of the standards in the sample spectrum. Only the phospholipid species displaying an intensity of at least 5 times the blank value were taken into account. To quantify the total amount of phospholipids in a phospholipid class, the abundances of individually measured species within the phospholipid class were totalled. Data were normalized on the basis of DNA amount. MRM-based analysis of the C16:1-containing phospholipids was based on a previous method.

**Analysis of lipid peroxidation sensitivity.** HUH7 control and FADS2 knockdown cells (105 per mL) (each) were seeded in 15-cm Petri dishes in 1% FBS DMEM. After 24 h, cells were treated with control or 5 μM RSL3 (the latter inhibits glutathione peroxidase 4 and induces lipid peroxidation). Lipid peroxidation was quantified using the MDA assay kit (Sigma) according to manufacturer’s instructions with some exceptions. In brief, 3 × 105 cells were collected in BHT-supplemented PBS. TBA-acetic acid solution was buffered to pH 3.5. Plates were read using an EnSpire Multimode Plate Reader (PerkinElmer). Signal was normalized to total amount of sample DNA.

**Analysis of membrane fluidity.** HUH7 control and FADS2 knockdown cells were grown on glass cover slips (n = 4) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549) for 3 days and subsequently fixed for 15–30 min in 4% PFA at room temperature. For lipid phase analysis, cells were stained with di-4-ANEPPDHQ (Thermo Fisher Scientific) according to the manufacturer’s specifications. For imaging, a Nikon A1R confocal microscope attached to Ti eclipse outfitted with the spectral detector was set to 530–590 nm and a Plan Apo VC 60× water immersion objective (Nikon Instruments) was used (Nikon Instruments). The spectral detector was set to 530–590 nm and 590–650 nm to image the spectral shift of the dye from lipid ordered to disordered phases. Resulting images were analysed with NIS software (Nikon Instruments), thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio. The higher the ordered-to-disordered ratio, the more saturated thereby segmenting the cells in the images and calculating the ordered-to-disordered ratio.
After 8 d, mice were either treated with vehicle (10% v/v ethanol, 40% v/v solubil) alone or with 1.5 mg per kg SCD inhibitor Merck Frosst compound 3) for six consecutive days twice daily p.o. Fourteen hours after the last treatment, mice were euthanized, the tumour nodule was resected, and blood and normal liver tissue were sampled. For metabolomic analysis, half of the tumour nodule and non-tumour tissues were rapidly frozen using a liquid-nitrogen-cooled Biosqueezer (Biospec Products). Tissues were then weighed (5–10 mg) and pulverized (Cryomill, Retsch) under liquid nitrogen conditions. The pulverized tissues were extracted for GC–MS analysis as described above. For histological quantification of the tumour area, half of the nodule was formalin-fixed and paraffin-embedded (FFPE). From these FFPE tissue blocks, 4-μm sections were cut and stained with H & E. The mean tumour area as a per cent of the total tissue area was determined in low-power magnifications by two independent physicians trained in histopathology. Humane endpoints were determined as a tumour size of 2 cm³. Additionally, the following symptoms were monitored and upon detection of one of the symptoms the mouse was euthanized: loss of ability to ambulate, laboured respiration, surgical infection or weight loss over 10% of initial body weight. Housing and experimental mouse procedures were approved by the Institutional Animal Care and Research Advisory Committee of KU Leuven.

Collection of clinical samples. Human samples were collected upon ethical approval of local authorities. Analysis was performed blinded. Patient information is provided in Supplementary Table 2. Liver. Liver and/or liver cancer samples were obtained from Indivumed GmbH, from the Laboratory of Hepatology (Commissie Medische Ethiek UZ Leuven – KU Leuven) and the archive of the Institute of Pathology of the LMU Munich with approval of the ethics committee of LMU Munich (approval no. 307–16 UE), respecting patients’ rights. Blood samples from healthy volunteers and patients with HCC were collected in collaboration with the Laboratory of Hepatology (UZ Leuven – KU Leuven) after obtaining informed consent. Freshly isolated primary hepatocytes (donor F125) were obtained from the Hepatocytes and Hepatic Stem Cells Bank from the Cliniques Universitaires St Luc. An agreement from the Belgian Ministry of Health was obtained for the Hepatocytes and Hepatic Stem Cells Bank. Written and signed informed consent has been obtained for collection of the cells.

Lung. Patients with non-small-cell lung cancer were enrolled in an IRB-approved protocol after obtaining informed consent (ClinicalTrials.gov identifier, NCT02095808). Study eligibility included pulmonary masses measuring 1 cm or more in diameter. Standard surgical procedures were followed, with the majority of cases being robotic lobectomies. On the basis of pre-operative imaging and gross inspection at resection, viable fragments of tumour and lung were sampled. Plasma samples from lung cancer patients were drawn primarily from an arterial line throughout the procedure.

Statistical analysis and software. Statistical data analysis was performed using GraphPad Prism 7 (GraphPad Software) on n ≥ 3 biological replicates. Details of statistical tests and post-tests are presented in the figure legends. Detection of mathematical outliers was performed using Grubb’s test. Sample size for all in vitro experiments was chosen empirically. For in vivo experiments, sample size was determined using power calculations with β = 0.8 and P < 0.05, based on preliminary data. Data are presented as mean ± s.d., or as mean ± s.e.m., as indicated in the figure legends.

Code availability. Custom code is available from the corresponding author upon reasonable request.

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

Data availability
The authors declare that all data supporting the findings of this study are available within the article, its Extended Data, Source Data or from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | SCD-independent cancer cells produce sapienate. a, Schematic overview of fatty acid metabolism. AcCoA, acetyl-coenzyme A; SCD1/5, stearoyl-CoA desaturase 1 and 5; Elovl5/6, elongation of very long chain fatty acids protein 5 and 6. b–e, SCD desaturation activity based on the palmitoleate-to-palmitate ratio, oleate-to-stearate ratio, and palmitoleate and palmitate synthesis upon treatment with Merck Frosst compound 3j (HUH7 and A549, 2 nM; H460 and DU145, 1 nM; MDA-MB-468 and T47D, 0.5 nM). b–d, n = 3; e, HUH7, n = 3; A459, n = 3; H460, n = 6 (control) and n = 4 (SCD inhibitor); DU145, n = 3; MDA-MB-468, n = 5; T47D, n = 5 (control) and n = 6 (SCD inhibitor)). Unpaired two-sided Student’s t-tests with Holm–Sidak multiple comparisons. f–h, Correlation between SCD independence and palmitate synthesis, growth rate or total fatty acid abundance (n = 3). SCD independence was defined as area under the cell number curve of Fig. 1a. Palmitate synthesis was derived from f. Total fatty acid abundance was derived from Extended Data Fig. 2a. Trend line (dashed line) and 95% confidence intervals (dotted lines) are depicted. Cancer cell experiments were performed in low FBS DMEM (1%, HUH7; 0.5%, others) with a treatment of 72 h. Error bars represent mean ± s.d. from biologically independent samples.
Extended Data Fig. 2 | Sapienate is produced via FADS2 in cancer cells. a, Heat map representing fatty acid abundances with or without treatment with Merck Frosst compound 3j (HUH7 and A549, 2 nM; H460 and DU145, 1 nM; MDA-MB-468 and T47D, 0.5 nM), normalized to the highest abundance of each fatty acid across all cell lines and conditions (Fig. 1b, Supplementary Table 1a). Over 90% reduction, white; no reduction, dark green. b, c, Desaturation activity to sapienate upon treatment with Merck Frosst compound 3j (HUH7 and A549, 2 nM; H460 and DU145, 1 nM; MDA-MB-468 and T47D, 0.5 nM; n = 3). Unpaired two-sided Student’s t-tests with Holm–Sidak multiple comparisons. d, Sapienate-to-palmitate ratio in HUH7 (n = 6) versus freshly isolated primary human hepatocytes (PHH; n = 3), DU145 (n = 6) versus RWPE-1 (n = 6) prostate cells, and MDA-MB-468 (n = 6) and T47D (n = 6) versus MCF10A (n = 6) breast cells. Unpaired Student’s t-tests and Welch’s correction (HUH7 versus PHH and DU145 versus RWPE-1); one-way ANOVA with Dunnett’s multiple comparisons (MDA-MB-468 and T47D versus MCF10A). e, Tumour weight of HUH7 subcutaneous xenografts treated with or without Merck Frosst compound 3j (n = 8, one experiment; 1.5 mg per kg twice daily p.o.). Unpaired Student’s t-test with Welch’s correction. f, g, FADS2 gene expression in cells with or without Merck Frosst compound 3j, as described in b and c, normalized to T47D cells (n = 3). One-way ANOVA with Tukey’s multiple comparisons (f); unpaired Student’s t-tests with Holm–Sidak multiple comparisons (g). h, FADS2 protein expression in the same conditions as in d. Statistics as described in d. n = 3. i, Expression of FADS2 gene or FADS2 protein in HUH7 and A549 cells upon FADS2 silencing, normalized to control (gene, HUH7, n = 3; A549, n = 6; protein, n = 3 (except for A549 shFADS2-2, n = 2)). One-way ANOVA with Dunnett’s multiple comparisons. Cancer cell experiments were performed in low FBS DMEM (1%, HUH7; 0.5%, others) with a treatment of 72 h. Error bars represent s.d. (in vitro) or s.e.m. (in vivo) from mean of biologically independent samples (in vitro) or mice (in vivo).
Extended Data Fig. 3 | Sapenate rather than arachidonate metabolism causes SCD independence.  

**a**, Relative FADS2 gene or FADS2 protein expression and desaturation activity to sapienate in MDA-MB-468 control and FADS2 overexpression cells with DMSO or 0.5 nM Merck Frosst compound 3j, normalized to control (n = 3). Unpaired two-sided Student’s t-test. 

**b**, Relative FADS2 gene expression in tumour nodules from HUH7 control or FADS2 knockdown orthotopic xenografts with vehicle or Merck Frosst compound 3j (1.5 mg per kg twice daily p.o.; n = 4; one experiment), normalized to control. One-way ANOVA with Tukey’s multiple comparisons. 

**c, d**, Relative desaturation activity from palmitate to sapienate or palmitoleate in normal adjacent liver (L) and tumour nodules (T), in the same model as described in **f**, normalized to normal liver controls. Control + vehicle (L), n = 18 (c) or n = 20 (d); control + vehicle (T), n = 18 (c) or n = 20 (d); control + SCD inhibition (L), n = 14 (c, d); control + SCD inhibition (T), n = 13 (c) or n = 14 (d); shFADS2-2 + vehicle (L), n = 19 (c, d); shFADS2-2 + vehicle (T) n = 18 (c, d); shFADS2-2 + SCD inhibition (L), n = 15 (c) or n = 16 (d); shFADS2-2 + SCD inhibition (T), n = 15 (c, d); two experiments. Two-way ANOVA with Sidak’s multiple comparisons. 

**e, f**, Desaturation activity from linoleate to γ-linolenate, on the basis of the γ-linolenate-to-linoleate ratio and arachidonate abundance in HUH7 and A549 control (non-targeting shRNA) and FADS2 knockdown (using one of two FADS2 shRNAs) cells (n = 3). One-way ANOVA with Dunnett’s multiple comparisons. 

**g, h**, Linoleate and arachidonate abundance in normal adjacent mouse liver and tumour nodules from HUH7 control (non-targeting shRNA) or FADS2 knockdown (shFADS2-2) orthotopic xenografts treated with vehicle or Merck Frosst compound 3j (1.5 mg per kg twice daily p.o.). Control + vehicle (L), n = 12 (g) or n = 14 (h); control + vehicle (T), n = 13 (g) or n = 14 (h); control + SCD inhibition (L), n = 14 (g) or n = 15 (h); control + SCD inhibition (T) n = 14 (g) or n = 16 (h); shFADS2-2 + vehicle (L), n = 14 (g) or n = 16 (h); shFADS2-2 + vehicle (T), n = 13 (g) or n = 15 (h); shFADS2-2 + SCD inhibition (L), n = 15 (g) or n = 18 (h); shFADS2-2 + SCD inhibition (T), n = 15 (g) or n = 16 (h); two experiments. Two-way ANOVA with Tukey’s multiple comparisons. Cancer cell experiments were performed in low FBS DMEM (1%, HUH7; 0.5%, others) with a treatment of 72 h. Error bars represent s.d. (in vitro) or s.e.m. (in vivo) from mean of biologically independent samples (in vitro) or mice (in vivo).
Extended Data Fig. 4 | Carbons from sapienate are detected in octadecenoate. a–f. 13C enrichment of palmitate or stearate from 13C6-glucose in HUH7 or A549 cells in control conditions (ethanol, black) or upon 13C-sapienate supplementation (blue). Cells were grown in 10% dialysed FBS DMEM containing 4.5 g per l 13C6 glucose for 1 week, after which cells were grown for 72 h in 0.5% FBS DMEM containing 4.5 g per l 13C6 glucose supplemented with ethanol or 20 μM 12C-sapienate. The purpose of this experiment was to trace the incorporation of carbons from sapienate into cis-8-octadecenoate. Palmitate and stearate were measured as controls. Because 13C-labelled sapienate is not commercially available, we performed a reverse labelling in which we pre-labelled HUH7 and A549 cells with 13C6-glucose to enrich cis-8-octadecenoate with 13C. Then, we supplemented these cells with unlabelled sapienate in the presence of 13C6-glucose and determined the 13C enrichment of octadecenoate. If sapienate is elongated to cis-8-octadecenoate, we expect a shift in the 13C enrichment from higher to lower octadecenoate isotopologues. We found that supplementation of unlabelled sapienate shifted the 13C enrichment accordingly (a, d). Moreover, the largest 13C enrichment increase was found in the M + 2 isotopologue, which indicates the elongation of unlabelled sapienate to octadecenoate with 13C-labelled acetyl-CoA. As expected, sapienate supplementation did not change (or only marginally changed) the 13C enrichment of palmitate and stearate (b, c, e, f). Unpaired two-sided Student’s t-tests; n = 3. Error bars represent mean ± s.d. from biologically independent samples.
Extended Data Fig. 5 | Sapienate is elongated to cis-8-octadecenoate.

a, Relative cis-8-octadecenoate abundance in cancer cells, normalized to T47D cells. HUH7, n = 3; A549, n = 3; H460, n = 5; DU145, n = 3; MDA-MB-468, n = 5; T47D, n = 5. One-way ANOVA with Tukey’s multiple comparisons. 
b, c, Relative cis-8-octadecenoate abundances in HUH7 and A549 control (non-targeting shRNA) and FADS2 knockdown (one of two FADS2 shRNAs) cells in control condition (ethanol) or upon supplementation with 20 μM sapienate, normalized to control. HUH7, control, n = 6 (ethanol) or n = 3 (sapienate); shFADS2-1, n = 3; shFADS2-2, n = 6 (ethanol) or n = 3 (sapienate); A549, control, n = 6; shFADS2-1, n = 3; shFADS2-2, n = 3. Data values are shown in Supplementary Table 1c, d. Two-way ANOVA with Tukey’s multiple comparisons. 
d, Relative proliferation of MDA-MB-468 cells with ethanol (n = 9) or 20 μM cis-8-octadecenoate (n = 3) upon treatment with DMSO or 0.5 nM Merck Frosst compound 3j. Data are normalized to control, with error bars representing s.e.m. Two-way ANOVA with Tukey’s multiple comparisons. 
e, f, Relative proliferation of HUH7 and A549 control (non-targeting shRNA) and knockdown (shFADS2) cells with ethanol or 20 μM cis-8-octadecenoate upon treatment with DMSO or 2 nM Merck Frosst compound 3j. HUH7, control, n = 9; shFADS2-1, n = 6; shFADS2-2, n = 9; A549; EtOH, n = 6; cis-8-C18:1, n = 3. Data are normalized to control. Two-way ANOVA with Tukey’s multiple comparisons. Only statistics for pairwise comparisons are depicted. Cancer cell experiments were performed in low FBS DMEM (1%, HUH7; 0.5%, all other cancer cells) with a treatment of 72 h. Error bars represent mean ± s.d. from biologically independent samples, unless otherwise noted.
Extended Data Fig. 6 | Sapienate and cis-8-octadecenoate are used in membranes. a–d, Heat map representing abundance changes of phosphatidylcholine (a), phosphatidylethanolamine (b), phosphatidylserine (c) and phosphatidylinositol (d) species in control and FADS2 knockdown HUH7 and A549 cells, relative to control. HUH7, control, n = 3; shFADS2-1, n = 4; shFADS2-2, n = 5; A549, n = 5. Only significant differences are depicted as fold change (log2(change with shRNA versus control)). X, blank or excluded values. Phospholipid species carrying sapienate or palmitoleate are depicted in bold red, and are listed in Supplementary Table 1e. Two-way ANOVA with Dunnett’s multiple comparisons. e, Relative distribution of phospholipid species in HUH7 (n = 2) and A549 (n = 5) cells with non-targeting shRNA (control). f, Membrane fluidity based on the ordered-to-disordered lipid ratio in HUH7 and A549 with a non-targeting shRNA (control, black) or one of two different shRNAs targeting FADS2 (brown (shFADS2-1) and orange (shFADS2-2)), normalized to control (n = 4). The higher the ordered-to-disordered ratio, the more saturated lipids are present in the membrane. One-way ANOVA with Dunnett’s multiple comparisons. g, Lipid peroxidation sensitivity via MDA assay in HUH7 with a non-targeting shRNA (control; black) or one of two different shRNAs targeting FADS2 (brown (shFADS2-1) and orange (shFADS2-2)) normalized to control (n = 3). Cells were treated with vehicle or 5 μM RSL3, which inhibits glutathione peroxidase 4 and induces lipid peroxidation. Two-way ANOVA with Sidak’s multiple comparisons. Cancer cell experiments were performed in low FBS DMEM (1%, HUH7; 0.5%, all other cancer cells) with a treatment of 72 h. Data are presented as mean ± s.d. from biologically independent samples.
Extended Data Fig. 7 | SCD independence and sapienate metabolism occur in medium with glucose and amino acid concentrations that are similar to physiological conditions. a, Fatty acid concentrations of fetal bovine serum (FBS; n = 4). Low FBS conditions (0.5–1% FBS) correspond to a total fatty acid concentration of 4.31–8.62 μM. b, Sensitivity profile of cancer cells to Merck Frosst compound 3j (white; HUH7 and A549, 2 nM; H460 and DU145, 1 nM; MDA-MB-468 and T47D, 0.5 nM) in BLM12,13, normalized to control. HUH7, n = 3; A549, n = 3; H460, n = 6; DU145, n = 6; MDA-MB-468, n = 3; T47D, n = 9. Two-way ANOVA with Dunnett’s multiple comparisons. c, d, Sensitivity profile of HUH7 and A549 control (non-targeting shRNA, black) and knockdown (shFADS2, brown (shFADS2-1) and orange (shFADS2-2)) cells treated with DMSO (dark bars) or 2 nM Merck Frosst compound 3j (light bars) in BLM12,13, normalized to control (n = 3). Two-way ANOVA with Holm–Sidak multiple comparisons. e, Sensitivity profile of MDA-MB-468 control (black) and FADS2 overexpression (green) cells treated with DMSO (dark bars) or 0.5 nM Merck Frosst compound 3j (light bars) in BLM12,13, normalized to control (n = 3). Two-way ANOVA with Holm–Sidak multiple comparisons. f, Desaturation activity to sapienate based on the sapienate-to-palmitate ratio in cancer cells, in conditions as described in b, n = 3. Unpaired two-sided Student’s t-tests with Holm–Sidak multiple comparisons. g, h, Desaturation activity from palmitate to sapienate based on the sapienate-to-palmitate ratio, in the same conditions as described in c–f, n = 3. One-way ANOVA with Dunnett’s multiple comparisons (g); unpaired Student’s t-test (h). Cancer cell experiments were performed in low FBS BLM (1%, HUH7; 0.5%, all other cancer cells) with a treatment of 72 h. Data are presented as mean ± s.d. from biologically independent samples.
Extended Data Fig. 8 | Separation and detection of sapienate and cis-8-octadecenoate. a, Separation and detection of sapienate (cis-6-hexadecenoate) and palmitoleate (cis-9-hexadecenoate) via GC–MS. Separation was optimized using a standard mix, containing pentadecanoate, sapienate, palmitoleate, palmitate, cis-8-octadecenoate, oleate, vaccenate, linoleate and stearate (top), and the method was subsequently validated by measurement of these fatty acids in biological samples. Bottom, a representative biological sample. b, Separation and detection of cis-8-octadecenoate, oleate (cis-9-octadecenoate) and vaccenate (cis-11-octadecenoate) via GC–FID. Separation was optimized using a standard mix containing cis-8-, cis-9- and cis-11-octadecenoate (top), and the method was subsequently validated by measurement of these fatty acids in biological samples. Bottom, a representative biological sample.
Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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☑️ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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☑️ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection  GC-MS data were collected using MSD Chem Station Data Acquisition (v6.02.02.1431) or MassHunter Acquisition (v8.08.02 Build 8.2.8260.0) software. Proteomic data were collected using Q Exactive HF Tune 2.9.

Data analysis  GC-MS data were analyzed using MSD Chem Station Data Analysis (v6.02.02.1431) or Agilent MassHunter (v8.08.02 Build 8.2.8260.0) software followed by an in-house developed Matlab script. Proteomic data were analyzed using MaxQuant (v1.6.1.0). Microsoft Excel 2013 was used for data output. Statistical data analysis was performed using GraphPad Prism 7 software.

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Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that all data supporting the findings of this study are available within the article, its extended data files, or from the corresponding author upon reasonable request.

Field-specific reporting

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Life sciences  Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/reportingsummary-flat.pdf

Life sciences study design

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

- Sample size: In vitro sample sizes were based on previous similar studies that have given statistical results. For in vivo experiments, sample size was determined using power calculations with β=0.8 and P<0.05, based on preliminary data and respects the limited use of animals in line with the 3R system: Replacement, Reduction, Refinement.
- Data exclusions: No data were excluded from the study.
- Replication: All experiments were carried out at least in triplicates. All attempts at replications were successful.
- Randomization: Mice were randomized into control and treatment groups. Human participants were allocated to healthy, HCC or LC patient cohorts, based on health status of the patient.
- Blinding: Mice and human participants were given a number (i.e., mouse XX and patient YY) prior to data collection and analysis. Data was collected and analyzed, and subsequently grouped in the corresponding cohorts for statistical analysis.

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 |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Human HEK293T epithelial cells, RWPE-1 prostate cells, MCF10A breast cells, A549 and H460 lung carcinoma, MDA-MB-468 and T47D breast adenocarcinoma, and DU145 prostate carcinoma cell lines were obtained from ATCC (Manassas, VA, USA), HUH7 liver carcinoma cell line was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan).

Authentication

MCF10A and MDA-MB-468 cells were authenticated via fingerprinting. All other cell lines used in this study were obtained from well-established cell banks (ATCC and JCRB), and therefore, no authentication was performed on these cells.
Mycoplasma contamination: All cell lines used in this study were tested negative for mycoplasma contamination.

Commonly misidentified lines: No commonly misidentified cell lines were used.

### Animals and other organisms

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

**Laboratory animals**

1. Subcutaneous Huh7 xenograft model: 8-9 week-old female immunocompromised NMRI nu/nu mice (Taconic M&B AS, Denmark) were injected and sacrificed after 2-3 weeks.
2. DEN-induced HCC model: 2 week-old male C57BL/6N mice (KU Leuven Animal Facility) were injected and sacrificed after 20-32 weeks.
3. Pten and Sh HCC model: male C57BL/6 mice (Institutional Animal Care at MD Anderson Cancer Center) were sacrificed after 12 months (Pten) or 4-6 months (Sh).
4. MyrAKT-N-Ras HCC model: male 6-8 week-old FVB/N mice (Committee for Animal Research, University of California) were injected and sacrificed after 6-9 weeks.
5. Orthotopic Huh7 HCC model: 6 week-old male immunocompromised NMRI nu/nu mice (Taconic M&B AS, Denmark) were injected and sacrificed after 2-3 weeks.

**Wild animals:** The study did not involve wild animals.

**Field-collected samples:** The study did not involve samples collected from the field.

### Human research participants

Policy information about studies involving human research participants.

**Population characteristics**

1. Blood samples: Healthy participants were 24-59 years old and this patient cohort contained 52% females and 48% males. HCC patients (diagnosed by a clinician) were 34-83 years old and this patient cohort consisted of 37.5% females and 62.5% males. Lung cancer patients (diagnosed by a clinician) were 43-85 years old and the cohort contained 66.5% females and 33.5% males.
2. Tissue samples: Liver and HCC samples were obtained via biobanks and did not involve the handling of human patients. Lung cancer and adjacent lung tissue samples involved handling of human patients, which were 55-85 years old. This cohort consisted of 60% females and 40% males.

**Recruitment**

Healthy participants were recruited via email inquiry and upon informed consent. Cancer patients were recruited through the clinicians based on the presence of HCC or lung cancer, respectively, upon informed consent. Additionally, retrospective analysis of irreversibly anonymized archival tissue samples no longer required for diagnostics was carried out with approval by the local authorities. Healthy volunteers were on average younger than cancer patients this could lead to a bias due to age related effect. Moreover, normal tissue was either adjacent to cancer tissue or from healthy individuals. Thus, the health status of the individual could create a bias.