Evidence for an alternative fatty acid desaturation pathway that increases metabolic plasticity in cancer cells

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Most tumors have an aberrantly activated lipid metabolism, which allows them to synthesize, elongate and desaturate fatty acids to support proliferation. However, targeting this metabolic liability only impacts some cancers suggesting that many cancer cells have an unexplored plasticity in their lipid metabolism. Here, we discover that cancer cells can exploit an alternative fatty acid desaturation pathway. We identify various cancer cell lines, murine hepatocellular carcinoma, and liver and lung cancers from patients that desaturate palmitate to the unusual fatty acid sapienate, normally produced by skin sebaceous gland cells. We observe that cancer cells synthesize sapienate to support membrane biosynthesis during proliferation. Sapienate synthesis therefore allows cancer cells to by-pass the known stearoyl-CoA desaturase (SCD)-dependent fatty acid desaturation. Accordingly, only targeting both desaturation pathways impairs proliferation of sapienate-synthesizing cancer cell lines *in vitro* and *in vivo*. In conclusion, we provide evidence that sapienate metabolism increases the metabolic plasticity of cancer cells.

INTRODUCTION

Metabolic rewiring is a hallmark of cancer cells that can be targeted for therapy[1-3]. Cancer cells often rewire metabolic pathways providing biosynthetic precursors that sustain uncontrolled proliferation[4-6]. Accordingly, increased lipid metabolism, including fatty acid synthesis, elongation and desaturation, is a metabolic alteration found in most proliferating cancer cells[2,7]. Surprisingly, however, only particular subsets of cancer cells are sensitive towards approaches targeting fatty acid metabolism, and in particular fatty acid desaturation[8-10]. This clearly separates fatty acid metabolism from other biosynthetic pathways such as nucleotide metabolism[3,11]. Yet, a mechanistic understanding of this unexpected observation remains elusive.

The well-characterized *de novo* fatty acid synthesis depends on acetyl-CoA to synthesize fatty acids of different length, most predominantly the sixteen-carbon-long palmitate[7]. Subsequently, palmitate is further converted through desaturation and elongation (Figure **1A**), which promotes its use in membrane synthesis needed for proliferation[10]. A known process that allows some cancer cells to bypass *de novo* fatty acid synthesis is the uptake of dietary fatty acids[12,13]. Yet, independent of the source of fatty acids, cancer cells often need to elongate and desaturate them to produce different lipid species[14]. Thus, inhibiting fatty acid desaturation should have detrimental effects on the proliferation of many cancer cells[15]. In contrast to this expectation, multiple studies show that even in conditions with low extracellular fatty acid availability, cancer cells display only a limited dependence on the fatty acid desaturation enzymes stearoyl-CoA desaturase (SCD) 1 and 5[12,16]. Collectively, these observations suggest the existence of an unexplored metabolic plasticity in the fatty acid desaturation metabolism of cancer cells. However, the nature of this metabolic plasticity is unknown.

Here, we discover an alternative fatty acid desaturation pathway novel to cancer cells. Specifically, we find that a significant number of cancer cells rewire their *in vitro* and *in vivo* fatty acid metabolism by desaturating palmitate to the unusual fatty acid sapienate. Our discovery explains metabolic plasticity in fatty acid metabolism and constitutes an unexplored metabolic rewiring in cancers.

RESULTS

Cancer cells differentially depend on the stearoyl-CoA desaturase pathway

Cancer cells display plasticity in their use of the SCD-dependent fatty acid desaturation pathway[12,16]. To mechanistically understand the nature of this metabolic plasticity we evaluated the dependence of different cancer cells on SCD activity. Specifically, we selected cancer cells that had been used in previous SCD pathway studies[17-20], and represent cancers of different origin (liver, lung, prostate, breast). We treated these cancer cells with the inhibitor Merck Frosst Cpd 3j[21] (0.5 – 2 nM), which specifically targets the human occurring isoforms SCD1 and 5[22], in conditions of low extracellular fatty acid availability. Subsequently, we determined sensitivity of the cancer cells to SCD inhibition based on cell number. Strikingly, we observed that cancer cells showed a broad sensitivity profile upon SCD inhibition ranging from unperturbed proliferation over impaired proliferation to cell death (Figure **1B**). Based on their proliferation response, we classified the cancer cells into SCD-dependent (proliferation inhibition or cell death; T47D, MDA-MB-468),

partially SCD-dependent (more than 50% impaired proliferation; DU145, H460), or SCD-independent (less than 50% impaired proliferation; A549, HUH7).

Next, we asked whether the differential dependency of cancer cells on SCD activity is caused by the degree with which Merck Frosst Cpd 3j inhibits their individual SCD activity. SCD enzymes catalyze the desaturation of palmitate and stearate to the monounsaturated palmitoleate and oleate, respectively (Figure **1A**)[23]. Thus, we assessed SCD activity upon Merck Frosst Cpd 3j treatment by measuring the ratio of palmitoleate to palmitate and oleate to stearate (using metabolomics), as well as palmitoleate synthesis (using ^{13}C tracer analysis[24,25]). Palmitate synthesis was measured as a control. If Merck Frosst Cpd 3j inhibits SCD activity, we expect that palmitoleate synthesis and the ratio between these fatty acids decreases, while palmitate synthesis remains unaltered. We observed that in all cell lines both the palmitoleate to palmitate and oleate to stearate ratios significantly decreased (Figure **1C**, **D**). In line, palmitoleate synthesis decreased in all cell lines, while palmitate synthesis did not change or changed to a lesser extent (Figure **1E**, **F**). Thus, we concluded that the desaturation activity of SCD was impaired to a similar extent across the different cancer cell lines, despite their differential proliferation response.

Cancer cells that synthesize high amounts of fatty acids or display increased proliferation might require more desaturation activity and thus could be more vulnerable to SCD inhibition. Therefore, we next asked whether the basal ability of cancer cells to synthesize palmitate or their basal growth rate was responsible for the observed differential SCD-dependence. Thus, we compared the cancer cell specific palmitate synthesis and growth rate in control condition to their proliferation response upon SCD inhibition. Yet, we did not find any significant correlation (Figure **1G, H**). Thus, we concluded that neither basal proliferation nor palmitate synthesis were the reason for the differential SCD-dependence of the cancer cells.

These collective data show that some cancer cells are SCD-independent and that this observation cannot be explained by their known metabolism.

Stearoyl-CoA desaturase-independent cancer cells synthesize sapienate

We hypothesized that SCD-independent and partially SCD-dependent cancer cells exploit an alternative desaturation pathway that allows them to proliferate upon SCD inhibition. We reasoned that the presence of such an alternative pathway must result in the synthesis of unusual monounsaturated fatty acids. Thus, we performed an untargeted mass spectrometry analysis of C12 to C18 saturated and monounsaturated fatty acids. The different cancer cells presented a

wide range in total abundance of C12 to C18 fatty acids, but this measure did not correlate with SCD independence (Extended Data Figure **1;** Extended Data Table 1 and 2). Yet, we strikingly discovered that SCD-independent and partially SCD-dependent cancer cells displayed an increased abundance of the unusual fatty acid sapienate (cis-6-C16:1) (Figure **2A;** Extended Data Table **1**). Moreover, we found that sapienate abundance increased upon SCD inhibition (Figure **2A**). Interestingly, sapienate is a major component of the human sebum and is to date considered to be a specific marker of sebocytes, which are the sebum-synthesizing cells of the sebaceous glands[26,27]. Thus, we concluded that, besides sebocytes, also cancer cells can synthesize sapienate. Since sapienate is a monounsaturated derivative of palmitate, we next determined the desaturation activity from palmitate to sapienate by assessing the sapienate to palmitate ratio and sapienate biosynthesis. We observed that the palmitate to sapienate ratio correlated with SCD independence (Figure **2B**) and that upon SCD inhibition the palmitate to sapienate ratio and sapienate biosynthesis increased (Figure **2C, D**). Taken together, these data indicate that sapienate is part of an alternative mono-desaturation pathway in cancer cells.

Next, we addressed whether cancer cells synthesize sapienate *in vivo*. To do so, we subcutaneously implanted hepatocellular carcinoma (HCC)-derived HUH7 cells (which showed the highest sapienate biosynthesis *in vitro*) into nude mice and dosed mice with Merck Frosst Cpd 3j (1.5 mg per kg; twice daily per oral). Subsequently, we measured the desaturation activity in the tumor xenografts based on the ratio between sapienate and palmitate. In accordance with our *in vitro* data, we observed that SCD inhibition in HUH7 xenografts did not significantly decrease final tumor weight, but significantly increased the desaturation activity to sapienate (Figure **2E, F**). Next, we addressed whether also carcinogen (diethylnitrosamine)- and genetically-induced murine HCC show desaturation activity to sapienate. Strikingly, we observed that also these tumors exhibited a significantly increased desaturation activity to sapienate compared to healthy liver (Figure **2G, H**). These data collectively show that cancer cells and in particular HCC produce sapienate both *in vitro* and *in vivo*.

It has been recently shown that the cellular origin of cancer cells can dictate the metabolic pathway spectrum they can rely on[2,28-31]. Therefore, we addressed the question whether cancers cells reflect the ability of non-transformed cells to produce sapienate. Thus, we assessed the sapienate to palmitate ratio in primary human hepatocytes (representing the origin of HCC[32,33]) and in immortalized non-transformed prostate (RWPE-1) and breast (MCF10A) cells. We found that primary human hepatocytes, RWPE-1 prostate cells and MCF10A breast cells display a low sapienate to palmitate ratio in the range of MDA-MB-468 and T47D breast cancer cells (Figure

2I), indicating a low desaturation activity to sapienate. These data suggest that liver and prostate cancer cells have an increased ability to produce sapienate compared to non-transformed cells of the same organ origin.

Sapienate is synthesized by fatty acid desaturase 2

In sebocytes, sapienate is produced by fatty acid desaturase (FADS) 2 from palmitate[26] (Figure **1A**). Therefore, we investigated whether cancer cells exploit FADS2 to synthesize sapienate. Accordingly, we found that *FADS2* gene expression was increased in SCD-independent and partially SCD-dependent cancer cells (Figure **3A**), and increased in liver and prostate cancer cells *in vitro* (HUH7, DU145) and *in vivo* (HUH7) upon SCD inhibition (Extended Data Figure **2A**, Figure **3B**). In line, FADS2 protein expression correlated with SCD independence and desaturation activity to sapienate in cancer cells (Figure **3C** and **3D**). Moreover, FADS2 protein expression was significantly higher in HUH7 and DU145 cancer cells compared to primary hepatocytes and non-transformed prostate (RWPE-1) cells (Figure **3E**). Accordingly, *FADS2* gene expression was increased in matched pairs of cancer versus non-cancerous tissue of HCC (three out of four) and lung cancer (XXXXX out of XXX) patients (Figure **3F**, **G**). These data suggest an involvement of FADS2 in sapienate biosynthesis.

To causally link FADS2 expression to sapienate biosynthesis, we generated FADS2 knockdowns in SCD-independent HUH7 and A549 cancer cells (Extended Data Figure **2B** and **2C**) and analyzed their desaturation activity to sapienate based on the sapienate to palmitate ratio. We found that FADS2 knockdown resulted in a significant decrease in desaturation activity to sapienate (Figure **3H**). Next, we asked whether also *in vivo* sapienate is synthesized via cancer cell intrinsic FADS2. Therefore, we implanted HUH7 control and FADS2 knockdown cells orthotopically into the liver of mice and measured the desaturation activity to sapienate in the arising liver tumors based on the sapienate to palmitate ratio. We observed that only FADS2 expressing tumors exhibited an increased desaturation activity to sapienate compared to healthy liver, while there was no significant difference between healthy liver and tumor upon FADS2 knockdown (Figure **3I**). Taken together, these findings show that sapienate is produced *in vitro* and *in vivo* via FADS2.

Sapienate biosynthesis causes stearoyl-CoA desaturase-independence

Next, we investigated whether sapienate biosynthesis causes SCD-independence. To do so, we overexpressed FADS2 in SCD-dependent MDA-MB-468 cells (Extended Data Figure **3A** and **3B**) and treated them with Merck Frosst Cpd 3j. We found that FADS2 overexpression resulted in an increased desaturation activity to sapienate (Figure **4A**) and a restored proliferation upon SCD inhibition, i.e. SCD-independence (Figure **4B**). Next, we assessed proliferation of the HUH7 and A549 FADS2-knockdown cells upon Merck Frosst Cpd 3j treatment. Sole FADS2 knockdown increased proliferation (Figure **4C, D**) indicating that cancer cells rely on the metabolic plasticity provided through simultaneous SCD and FADS2 desaturation activity in the expense of maximized proliferation. Accordingly, FADS2 knockdown combined with SCD inhibition resulted in proliferation inhibition in HUH7 cells and cell death in A549 cells (Figure **4C, D**). Subsequently, we assessed dual inhibition of SCD- and FADS2-dependent desaturation *in vivo* in orthotopically implanted HUH7 (control and FADS2 knockdown) liver tumors. We assessed tumor area in the arising liver nodules upon vehicle or Merck Frosst Cpd 3j treatment (1.5 mg per kg; twice daily per oral) using hematoxylin and eosin staining. We found that only dual inhibition of SCD and FADS2 resulted in a significantly smaller tumor area compared to control tumors (Figure **4E** and **4F**). Differently to the *in vitro* results no full inhibition of tumor growth was achieved *in vivo*, likely due to the *in vivo* knockdown efficiency of 62.5% and a partial compensation through extracellular sapienate uptake (Extended Data Figure **3C-E**). Taken together, these data demonstrate that dual activity of SCD- and FADS2-dependent desaturation provides metabolic plasticity supporting proliferation, which is abolished *in vitro* and *in vivo* upon inhibition of both pathways.

FADS2 is also known to desaturate the essential fatty acid linoleate to γ-linolenate yielding arachidonate as final product[34]. Thus, we investigate the possibility that FADS2 expression provides metabolic plasticity through the synthesis of polyunsaturated fatty acids rather than sapienate. To do so, we measured FADS2 activity in poly-desaturation based on the γ-linolenate to linoleate ratio as well as linoleate and arachidonate abundance upon FADS2 knockdown *in vitro* and *in vivo*. Yet in contrast to this possibility, we found that neither of these measures significantly changed in FADS2 knockdown cancer cells compared to control *in vitro* and *in vivo* (Figure **4G-J**). This suggests that desaturation of linoleate by FADS2 is not causal for SCD-independence. Yet, supplementation of sapienate was sufficient to rescue proliferation of SCD-dependent MDA-MB-468 cells upon Merck Frosst Cpd 3j treatment (Figure **4K**). Moreover, the proliferation defect observed by dual inhibition of FADS2 and SCD desaturation activity in HUH7 and A549 cells was (partially) rescued by supplementation of sapienate or the SCD product palmitoleate (Figure **4C, D**). Based on these data we concluded that desaturation of palmitate to sapienate, rather than desaturation of linoleate, is causal for SCD-independence.

Sapienate is elongated and used for membrane synthesis

Next, we asked how sapienate supports cancer cell proliferation. Since an important fate of fatty acids is membrane synthesis, we investigated whether sapienate is elongated and incorporated into membranes. The theoretical elongation product of sapienate is cis-8-octadecenoate (cis-8-C18:1) (Figure **1A**). Thus, we first assessed cis-8-octadecenoate abundance in the cancer cells and found that the abundance of cis-8-octadecenoate was more than 5-fold higher in SCD-independent cells than in SCD-dependent cells (Figure **5A**). Accordingly, cis-8-octadecenoate abundance increased upon sapienate supplementation, while FADS2 knockdown resulted in decreased cis-8-octadecenoate abundance (Figure **5B, C**; Extended Data Table **3** and **4**). In line, the relative cis-8-octadecenoate (and sapienate) abundance increase matched the relative oleate (and palmitoleate) abundance decrease upon SCD inhibition in HUH7 and A549 cells (Figure **5D, E** and Extended Data Table **3** and **4**). This suggests that cis-8-octadecenoate (and sapienate) can replace oleate (and palmitoleate) upon SCD inhibition. Consistently, we found that cis-8-octadecenoate supplementation rescued the proliferation of SCD-dependent MDA-MB-468 cells and FADS2-knockdown cells (HUH7, A549) upon Merck Frosst Cpd 3j treatment (Figure **5F-H**). Notably, cis-8-octadecenoate (Figure **5G, H**) was more potent than either palmitoleate or sapienate (Figure **4C, D**) in rescuing the proliferation of FADS2 knockdown cells, indicating an importance of sapienate elongation for their proliferation.

Next, we traced the incorporation of carbons from sapienate into cis-8-octadecenoate. Palmitate and stearate were measured as controls. Since ^{13}C labeled sapienate is not commercially available, we performed a reverse labeling in which we pre-labeled HUH7 and A549 cells with $^{13}C_6$-glucose to enrich cis-8-octadecenoate with ^{13}C. Then, we supplemented these cells with unlabeled sapienate in the presence of $^{13}C_6$-glucose, and determined the ^{13}C enrichment of octadecenoate. If sapienate is elongated to cis-8-octadecenoate, we expect a shift in the ^{13}C enrichment from higher to lower octadecenoate isotopologues. Indeed, we found that supplementation of unlabeled sapienate shifted the ^{13}C enrichment accordingly (Figure **5I, J**). Moreover, the largest ^{13}C enrichment increase was found in the M2 isotopologue, indicating the elongation of unlabeled sapienate to octadecenoate with ^{13}C labeled acetyl-CoA. As expected, sapienate supplementation did not or only marginally change the ^{13}C enrichment of palmitate and stearate (Extended Data Figure **4A-D**). These collective data show that sapienate is elongated to cis-8-octadecenoate.

We then sought to address whether sapienate is used for membrane synthesis. To do so, we characterized the phospholipid composition of SCD-independent HUH7 and A549 control and FADS2 knockdown cells. We found that FADS2 knockdown altered the overall composition of membrane-bound phosphatidylcholines, phosphatidylethanolamines, phosphoinositols and phosphatidylserines (Extended Data Figure **4E-I**, Extended Data Table **5**). Accordingly, FADS2 knockdown decreased the fraction of phospholipids build from sapienate and increased phospholipids build from the SCD product palmitoleate (Figure **5K**). In line, SCD inhibition decreased the phospholipid-bound palmitoleate fraction, while the phospholipid-bound sapienate and cis-8-octadecenoate fraction increased (Figure **5L-M**). Functionally, these changes in membrane composition resulted in decreased membrane fluidity and increased resistance to lipid peroxidation in FADS2 knockdown cells (Extended Data Figure **4J, K**). Taken together, these data show that cancer cells can elongate sapienate and use it for membrane biosynthesis.

Sapienate biosynthesis occurs in human lung and liver cancers

Finally, we asked whether sapienate biosynthesis also occurs in human cancers. To address this question, we measured the ratio of sapienate to palmitate in cancer and healthy lung and liver tissue as well as blood plasma from humans. Healthy blood plasma was from volunteers, while healthy lung and liver was adjacent non-cancerous tissue from cancer patients and non-transplanted donor organs. In addition, we measured the palmitoleate to palmitate ratio in the same tissues and blood plasmas as a readout of the SCD-dependent fatty acid desaturation pathway. Strikingly, we found that only in cancer tissue, but not in healthy tissue, the sapienate to palmitate ratio was significantly increased compared to the blood plasma ratio (Figure **6A-B**). Accordingly, the sapienate to palmitate ratio was higher in cancer tissue compared to healthy tissue (Figure **6A-B**). Notably, 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 healthy tissue (Figure **6A-B**). This suggests a specific increase in sapienate biosynthesis in cancers, rather than a general increase in the synthesis of monounsaturated fatty acids. Taken together, these data demonstrate that sapienate biosynthesis occurs *in vivo* in lung and liver cancers from human patients.

DISCUSSION

So far, the well-characterized SCD-dependent fatty acid desaturation pathway was considered to be the only source of *de novo* generated monounsaturated fatty acids in cancer cells[35-37]. However, we discovered that cancer cells can rewire their fatty acid metabolism and desaturate palmitate to the unusual fatty acid sapienate. This alternative fatty acid desaturation pathway increases the metabolic plasticity of cancer cells and enables them to gain independence from the SCD-dependent fatty acid desaturation pathway. We find that sapienate biosynthesis is particular active in human lung and liver cancers. Our finding is novel and highly relevant because it explains the metabolic plasticity observed in the fatty acid desaturation metabolism of many human cancers. Therefore, it could constitute a biomarker for treatment stratification and a potential target for combination therapies. In this respect, Rees and colleagues showed that sensitivity to ML293, a compound previously shown to induce cytotoxicity in breast cancer stem cells[38], correlates with FADS2 expression[39]. Moreover, we show that sapienate is elongated into an unusual fatty acid cis-8-octadecenoate that has so far not been described in the context of cancer. While we find that sapienate and cis-8-octadecenoate supports membrane synthesis, it is tempting to speculate that sapienate and its elongation products impact the known fatty acid and lipid signaling networks of cancer cells[40-42]. Consequently, this could provide cancer cells with a so far unexplored possibility to deregulate signaling networks, opening new opportunities to understand and target them in cancers.

To date, sapienate biosynthesis is considered to be a highly specific marker of human sebocyte metabolism[27]. While sapienate biosynthesis can be induced in human cancer cells via genetic engineering[43], physiological sapienate biosynthesis has so far not been described in human or murine cancer cells. We demonstrate that sapienate is produced in both via FADS2. Differently to the known role of FADS2 in arachidonate production and poly-desaturation of essential fatty acids[7,44,45], we show that cancer cells can repurpose this enzyme (similarly to sebocytes) to synthesize sapienate. In our dataset, only breast cancer cells were unable to rely on sapienate biosynthesis. This finding could be explained by the observation that a subset of invasive breast cancers has lost the 11q13 locus that includes *FADS2*[46,47]. This indicates that in particular breast cancers have a low cell intrinsic plasticity in their fatty acid metabolism. Yet, as breast cancer cells are known to cooperate with adipocytes[48-50], which provide them with fatty acids[51], the presumable disadvantage of losing the 11q13 locus might be balanced. Interestingly, the 11q13 locus is amplified in many other cancers of different tissue origin[52-54], suggesting sapienate biosynthesis as a broad metabolic rewiring of human cancers.

In conclusion, with our discovery we increase the understanding of fatty acid metabolism in cancers and suggest sapienate biosynthesis as an important alternative source of monounsaturated fatty acids particular in human lung and liver cancers.

EXPERIMENTAL PROCEDURES

Cell lines, cell culture and chemicals

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). All cell lines were confirmed to be mycoplasma free based on the MycoAlert™ Mycoplasma Detection Kit (Lonza, Basel, Switzerland). 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 (50U per mL) and 1% streptomycin (15 µg per mL) (all Life Technologies, CA, USA). MCF10A cells were cultured in Dulbecco's modified Eagle's medium-F12 (DMEM-F12) (Life Technologies, CA, USA), supplemented with 5% horse serum (Life Technologies, CA, USA), 1% penicillin (50U per mL) (Life Technologies, CA, USA), 1% streptomycin (15 µg per mL) (Life Technologies, CA, USA), 0.5 µg per mL hydrocortisone (Sigma-Aldrich, MO, USA), 100 ng per mL cholera toxin (Sigma-Aldrich, MO, USA), 10 µg per mL insulin (Sigma-Aldrich, MO, USA), and 20 ng per mL recombinant human epidermal growth factor (PeproTech EC, London, UK). Other cells were cultured in high glucose (4.5 g per L) Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, MA, USA), 1% penicillin (50U per mL) (Life Technologies, CA, USA) and 1% streptomycin (15 µg per mL) (Life Technologies, CA, USA). For growth and labeling experiments, low serum conditions (0.5-1% FBS) were applied. $^{13}C_6$-glucose (CLM-1396 Cambridge Isotope Laboratories, MA, USA) was used for labeling experiments. Hygromycin B and puromycin dihydrochloride (Life Technologies, CA, USA) were added to the growth medium for selection of overexpression and knockdown cell lines, respectively. Merck Frosst Cpd 3j[21] was used as an SCD inhibitor, as described in the patent application WO2006/130986. Specificity of Merck Frosst Cpd 3j towards SCD1 and 5 was confirmed by displacement of radiolabeled-stearoyl-CoA from Sf9 membranes expressing recombinant SCD1 or 5. The fatty acids palmitoleate (16:1), sapienate (16:1) and oleate (18:1) were purchased from Sigma-Aldrich (MO, USA). Cis-8-octadecenoate (18:1) was purchased from Larodan (Solna,

Sweden). Solvents for metabolite extraction and mass spectrometry were HPLC grade from Sigma-Aldrich (MO, USA).

Knockdown and overexpression strategies

FADS2 knockdown cell lines were generated using the shRNA-expressing lentiviral pLKO1-puro vector with a puromycin selection cassette (Plasmid #8453; Addgene, MA, USA). Clone IDs for shRNAs were as follows: shFADS2-1 (TRCN0000064755) and shFADS2-2 (TRCN0000064757) (Sigma-Aldrich, MO, USA). A scrambled shRNA, *i.e. TRC1* was used as a negative control for *FADS2* knockdown cells. *FADS2* overexpression cell lines were generated using the pLVX-IRES-Hyg vector with a hygromycin selection cassette (Clontech Laboratories Inc., CA, USA). An empty pLVX-IRES-Hyg vector served as a negative control for *FADS2* overexpression. Lentiviruses were produced by transfection of HEK293T cells. Transduction of cells was performed overnight and medium was replaced the next day. Poly-clonal cells were selected for 1-2 weeks with puromycin in the case of knockdown cells or with hygromycin in the case of overexpression before experiments were performed. All knockdown and overexpression cell lines were validated by quantitative real-time PCR and proteomic analysis (Extended Data Figure **2C, D** and Extended Data Figure **3A, B**. Primers for *FADS2* were designed to amplify a cDNA segment in the sequence as follows: forward primer 5'-gaccacggcaagaactcaaag-3' and reverse primer 5'-gagggtaggaatccagccatt-3'. For *SCD1*, the forward and reverse primer used were 5'-tctctgctacacttgggagc-3' and 5'-gagctttgtaagagcggtgg-3', respectively. Relative gene transcript levels were compared to the control gene *RPL-19*, with 5'-attggtctcattggggtctaac-3' and 5'-agtatgctcaggcttcagaaga-3' as forward and reverse primer, respectively. Real-time PCR reactions were performed on a 7,500 Fast Real Time PCR System (Applied Biosystems, Life Technologies). Amplification was performed at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Samples were assayed in triplicates.

Quantification of *FADS2* gene expression in primary tumors

Human tissue samples were retrieved from the archive of the Institute of Pathology of the LMU Munich with approval of the LMU Munich's ethics committee (approval no. 307-16 UE). Representative tumor and normal tissue areas were reviewed by a resident pathologist in representative tissue slides stained with hematoxylin and eosin (H&E). Corresponding areas were micro-dissected from paraffin blocks using biopsy punches (2 mm diameter). Deparaffinization of the tissue and RNA isolation was carried out using the Formapure Total Kit (Beckman Coulter). cDNA synthesis was performed using 1 µg of RNA with the MultiScribe Reverse Transcription kit

(Applied Biosystems). Analysis of the mRNA expression of *FADS2* was performed by qRT-PCR using SYBR-Green Master Mix (Applied Biosystems) in a CFX Connect Real-Time System (Bio-Rad). Primers were used at a concentration of 0.5 µM in a final reaction volume of 15 µl. Three technical replicates were analyzed per sample. *RPLPO* expression was used as a control for normalization. Primer sequences were as follows: forward primer 5'-gaccacggcaagaactcaaag-3' and reverse primer 5'-gagggtaggaatccagccatt-3'.

Growth and labelling experiments

Cancer cells were seeded in the wells of 12-well plates (Corning, NY, USA) at either $7x10^4$ cells per well (HUH7, A549, H460 and DU145), $1.5x10^5$ cells per well (MDA-MB-468) or $2x10^5$ cells per well (T47D) in low FBS DMEM (1.5 mL per well) and grown in a humidified environment at 37°C with 5% CO_2. For HUH7, 1% FBS was considered low FBS DMEM; for all other cell lines 0.5% FBS was used. Low FBS (0.5-1%) DMEM contains a total of 4.31-8.62 µM fatty acids, with palmitate, oleate and stearate being the most abundant fatty acids (Extended Data Figure **5A**). After 24 hours, the medium was aspirated, cells were washed with DPBS, and low FBS DMEM (4.5 g per L glucose for growth experiments or 4.5 g per L $^{13}C_6$-glucose for labeling experiments, including fatty acid synthesis assessment) supplemented with 0.1% DMSO (control) or Merck Frosst Cpd 3j (either at 0.5 nM, 1 nM or 2 nM concentration dissolved in DMSO) was added to the wells (1.5 mL per well). Treatment was carried out for 72 hours, during which cells were grown in a humidified environment at 37°C with 5% CO_2. For growth experiments, cells were counted prior to treatment and 72 hours after treatment using a MoxiTM Z Mini Automated Cell Counter (Orflo Technologies, ID, USA) or a Countess™ II Automated Cell Counter (Thermo Fisher Scientific, MA, USA) following trypsinization. For labeling 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 a more physiological Blood-Like Medium[55,56] (BLM) with low FBS (Extended Data Figure **5B-I**).

For rescue experiments, cells were seeded and grown as described above. After 24 hours, the medium was replaced by medium containing 0.1% DMSO (control) or Merck Frosst Cpd 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-C18:1) dissolved in ethanol. Cells were counted prior to treatment and 72 hours after treatment using a MoxiTM Z Mini Automated Cell Counter (Orflo Technologies, ID, USA) or a Countess™ II Automated Cell Counter

(Thermo Fisher Scientific, MA, USA) following trypsinization. All experiments were performed in triplicates. Changes in intracellular fatty acid abundance upon supplementation were confirmed by mass spectrometry (Extended Data Table **3** and **4**).

Primary hepatocytes of one donor were obtained from the Hepatocytes and Liver Stem Cell Bank, Cliniques Universitaires St Luc, Brussels, Belgium. Cells were thawed using the Corning® Gentest™ High Viability CryoHepatocyte Recovery Kit (Corning, NY, USA). Cells were then plated on collagen I-coated 6-well plates at a density of $1.4x10^6$ cells per well, and left to attach for 8 hours in Corning Plating Medium (Corning, NY, USA).

Reverse labelling experiments

HUH7 and A549 cells were seeded at $1x10^6$ cells per flask in T75 flasks in low FBS (1% FBS, HUH7) or 10% FBS (A549) DMEM and grown as described above. After 24 hours, the medium was aspirated, cells were washed with DPBS and 1% (HUH7) or 10% (A549) dialyzed FBS DMEM (4.5 g per L $^{13}C_6$-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 $7x10^4$ cells per well in low FBS DMEM containing 4.5 g per L $^{13}C_6$-glucose (1% FBS for HUH7 and 0.5% FBS for A549). After 24 hours, the medium was aspirated, cells were washed with DPBS and low FBS DMEM containing 4.5 g per L $^{13}C_6$-glucose (1% FBS for HUH7 and 0.5% FBS for A549) supplemented with either 1% ethanol (control) or 20 µM ^{12}C sapienate (cis-6-16:1) dissolved in ethanol was added to the cells (1.5 mL per well). Treatment was carried out for 72 hours. After treatment, cells were washed with saline solution 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.

If fully labelled cells use ^{12}C sapienate, a decrease in the ^{13}C enrichment will be observed in the metabolites into which sapienate is incorporated, such as cis-8-octadecenoate. Since our GC-MS setup does not allow for separation of cis-8 and cis-9-octadecenoate, and our GC-FID setup does not allow for assessment of ^{13}C enrichment, we opted to assess the ^{13}C enrichment in the pool of octadecenoate (consisting of cis-8 and cis-9-octadecenoate) via GC-MS. Note that only cis-8-octadecenoate, and not cis-9-octadecenoate, can result from sapienate metabolism. Hence, a decrease in ^{13}C enrichment in the octadecenoate pool indicates incorporation of ^{12}C sapienate into cis-8-octadecenoate.

Metabolite extraction and metabolite measurement

Metabolite extractions were performed using the methods described in Christen *et al*. and Lorendeau *et al*. [57,58]. Briefly, for cell culture plates containing 100k-500k cells per well, medium was aspirated, cells were washed with blood bank saline and cell metabolism was quenched by flash-freezing the plates in liquid nitrogen. Next, 400 µL -20°C cold 65% methanol was added to the wells, cells were scraped with a pipet tip and suspensions were transferred to Eppendorf tubes. Next, 250 µL -20°C cold chloroform was added and samples were vortexed at 4°C for 10 min to extract metabolites. Phase separation was achieved by centrifugation at 4°C for 10 min, after which the chloroform phase (containing the total fatty acid content) was separated and dried by vacuum centrifugation. For tissue samples, tissues were weighed (5-10 mg) and pulverized (Cryomill, Retsch) under liquid nitrogen conditions. For plasma samples, 10 µL (murine) or 50 µL (human) of cold plasma was transferred to an Eppendorf tube. Next, 800 µL -20°C cold 65% methanol was added to the samples, followed by 500 µL -20°C cold chloroform. Samples were then handled as described above. Dried fatty acid samples were immediately processed to fatty acid methyl esters as described below, thereby avoiding degradation.

Total fatty acid samples were esterified with 500 µL 2% sulphuric acid in methanol for 180 min at 60°C or overnight at 50°C and extracted by addition of 600 µL hexane and 100 µL saturated NaCl. Samples were centrifuged for 5 min and the hexane phase was separated and dried by vacuum centrifugation. Samples were resuspended in hexane, after which isotopologue distributions of fatty acids were measured with a 7890A GC system (Agilent Technologies, CA, USA) combined with a 5975C or a 7000 inert MSD system (Agilent Technologies, CA, USA). One microliter of each sample was injected in splitless (5975C) or 5:1 split (7000) mode with an inlet temperature of 270°C onto a DB35MS column. Helium was used as a carrier gas with a flowrate of 1 mL min^{-1}. The oven was held at 80°C for 1 min and ramped with 5°C min^{-1} to 300°C. The MS system was operated under electron impact ionization at 70eV and a mass range of 100-650 amu was scanned. Evidence for separation of cis-6-C16:1 and cis-9-C16:1 is presented in Extended Data Figure **5J**. Isotopologue distributions were extracted from the raw chromatograms using an in-house Matlab script. Correction for naturally occurring isotopologues was achieved using Isocor software[59]. Palmitate, palmitoleate and sapienate synthesis were calculated based on isotopologue distributions of the according fatty acid, using Isotopomer Spectral Analysis (ISA)[60] using an in-house MATLAB script. For determination of relative metabolite abundances, the total ion counts were normalized to the internal standard (pentadecanoate or heptadecanoate) and the cell number or protein content for cell extracts. For tissue extracts, the total ion counts were normalized to the internal standard (pentadecanoate or heptadecanoate) and tissue weight. The desaturation activity from palmitate to sapienate or palmitoleate was calculated by dividing the

normalized total ion count of sapienate or palmitoleate by the normalized total ion count of palmitate.

Cis-8-octadecenoate abundances were measured with a gas chromatography flame ionization detector (GC-FID) system (TRACE GC ULTRA, Thermo Fisher Scientific Inc., Waltham, MA). One microliter of samples was injected with an inlet temperature of 250°C onto a SLB-IL111 capillary column (100 m x 0.25 mm ID, 0.20µm thickness, Supelco, Bellefonte, PA). Helium was used as a carrier gas at 1.2 mL min^{-1} and the oven temperature was maintained isothermally at 140°C. cis-C18:1 isomers (Δ8,9,11) were synthesized as described before[61], processed to fatty acid methyl esters and used for validation of the separation of cis-C18:1 isomers and confirmation of the retention times of respective cis-C18:1 fatty acid methyl esters. Evidence for separation of cis-8-C18:1 and cis-9-C18:1 is presented in Extended Data Figure **5K**. For determination of relative cis-8-octadecenoate abundances, the total ion counts were normalized to the internal standard (pentadecanoate or heptadecanoate) and the protein content or tissue weight for cell extracts or tissue extracts, respectively.

FADS2 proteomic analysis

(Francis)

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 hours and subsequently grown for 72 hours in 1% FBS DMEM. Cells were trypsinized, washed with blood bank saline and re-suspended in TAG lysis buffer (1% IGEPAL® CA-630 (Nonidet P-40), 50 mM Trizma® hydrochloride and 150 mM sodium chloride). Lipids were extracted from cell lysate according to the Folch method[62]. An internal standard containing a known concentration of 1,2-Diheptanoyl-sn-glycero-3-phosphocholine (17:0) was added to samples prior to extraction to allow the quantification of total phospholipids. Lipid fractions were separated by thin-layer chromatography and fatty acid methyl esters (FAMEs) were prepared as previously described[63,64]. Separation and detection of total phospholipid FAMEs was achieved using a 6890N Network GC System (Agilent Technologies; CA, USA) with flame ionization detection. FAMEs were identified by their retention times compared to a standard containing 31 known fatty acids and quantified in micromolar from the peak area based on their molecular weight. The micromolar quantities were then totaled and each fatty acid was expressed as a percentage of this value (molar percentage; mol%) or µg fatty acids normalized to cellular protein concentration.

Analysis of phospholipid species

HUH7 and A549 cells carrying a non-targeting shRNA or a shRNA targeting FADS2 (shFADS2-1 and shFADS2-2) were seeded in T75 flasks, allowed to attach for 24 hours and subsequently grown for 72 hours 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. 0.7 mL of homogenized cells were mixed with 0.9 mL MeOH:HCl (1N) (8:1), 0.8 mL $CHCl_3$ 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 spd111v (Thermo Fisher Scientific) at room temperature and the remaining lipid pellet was stored at −20°C under argon. Prior to mass spectrometry analysis, lipid pellets were reconstituted in running solution (CH_3OH:$CHCl_3$:NH_4OH; 90:10:1.25; v/v/v). Lipid standards PC25:0, PC43:6, SM30:1, PE25:0, PE43:6, PI25:0, PI31:1, PI43:6, PS25:0, PS31:1 and PS37:4 (Avanti Polar Lipids) were added based on the amount of DNA of the original sample. Phospholipids were analyzed by electrospray 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 (Advion Biosciences) for automated sample injection and spraying as described[65]. Intact phospholipid species were analyzed as described by Rysman *et al*[65]. Phospholipid profiling was executed by (positive or negative) precursor ion or neutral loss scanning at a collision energy of 50 eV/45 eV, 35 eV, −35 eV and −60 eV for precursor 184 [sphingomyelin (SM)/phosphatidylcholine (PC)], neutral loss 141 [phosphatidylethanolamine (PE)], neutral loss 87 [phosphatidylserine (PS)] and precursor 241 [phosphatidylinositol (PI)], respectively. Phospholipid quantification was performed by multiple reaction monitoring (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). Only the phospholipid species displaying an intensity of at least 5 times the blank value were taken into account. In order to quantify the total amount of phospholipids in a phospholipid class, we summed the abundances of individually measured species within the phospholipid class. Data were normalized based on the amount of DNA. The composition of C16:1-containing phospholipid species was analysed as described by Ekroos *et al*[66].

Analysis of lipid peroxidation sensitivity

Thirty million HUH7 control and FADS2 knockdown cells were seeded in 15-cm Petri dishes in 1% FBS DMEM. After 24 hours, cells were treated with control or 5 µM RSL3, the latter inhibiting

glutathione peroxidase 4 and inducing lipid peroxidation. Lipid peroxidation was quantified using the MDA assay kit (Sigma) according to manufacturer's instructions with some exceptions. Briefly, 30 million 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 and A549 control and FADS2 knockdown cells were grown on glass coverslips (n=4) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549) for 3 days and subsequently fixed for 15-30min in 4% PFA at room temperature. For lipid phase analysis, cells were stained with di-4-ANEPPDHQ (Thermo Fisher Scientific, MA, USA) according to the manufacturer's specifications. For imaging, a Nikon A1R confocal microscope attached to Ti eclipse outfitted with a Plan Apo VC 60x lens oil immersion lens with an NA of 1.4 was used (Nikon Instruments, NY, USA). The spectral detector was set to 530-590 and 590-650 nm to image the spectral shift of the dye from lipid ordered to disordered phase. Resulting images were analyzed with NIS software (Nikon Instruments, NY, USA), thereby segmenting the cells in the images and calculating the ordered to disordered ratio[67]. The higher the ordered to disordered ratio, the more saturated lipids are present in the membrane. Per coverslip, 10 fields of views were imaged.

Mouse models

Subcutaneous HUH7 xenograft model: Mice were fed *ad libitum* a CRM (E) expanded low fat diet (Special Diets Services 801730) one week prior to the start of the experiments until sacrificing. $1x10^6$ HUH7 cells were subcutaneously injected in 50% Matrigel™, (BD Biosciences), 50% cell culture medium without FCS into 8-9-week old female immunocompromised NMRI$^{nu/nu}$ mice (Taconic M&B AS, Ejby, Denmark). Tumors were allowed to grow up to a mean tumor size of 30 mm^2 (length x width), before mice were allocated to treatment and control group by stratified randomization based on their primary tumor size (8 animals per group). Tumor-bearing mice were either treated with vehicle (10% v/v ethanol, 40% v/v solutol) alone or with 1.5 mg per kg SCD inhibitor Merck Frosst Cpd 3j for six consecutive days twice daily per oral (p.o.). After the last treatment, mice were euthanized and tumors were harvested and snap-frozen for further analyses. All animal experiments were conducted in accordance with the German animal welfare law and approved by local authorities.

DEN-induced hepatocellular carcinoma model: male C57Bl/6N mice were obtained from the KU Leuven animal facility and injected with diethylnitrosamine (DEN) (25mg per kg) intraperitoneally

at the age of 14 days. After 4 weeks, mice were fed with a 13kJ% fat diet (Ssniff S8655-E220) until sacrificed and analyzed at 20, 30, 31 or 32 weeks after DEN administration. Tumor and non-tumor tissues were collected and rapidly frozen for metabolomic analysis using a liquid nitrogen cooled Biosqueezer (Biospec Products). Tissues were weighed (5-10 mg) and pulverized (Cryomill, Retsch) under liquid nitrogen conditions. The pulverized tissues were extracted for GC-MS analysis as described above. Housing and experimental animal procedures were approved by the Institutional Animal Care and Research Advisory Committee of the University of Leuven, Belgium.

Hepatocyte-specific Pten *and* Stk *knockout hepatocellular carcinoma model:* mice were fed standard chow *ad libitum* throughout the procedure. C57BL/6 mice carrying *Pten* conditional knockout alleles were crossed with Albumin (Alb)-Cre-transgenic mice. Control animals were *Pten*$^{loxP/loxP}$; Alb-Cre^{-}, whereas the experimental mice were *Pten*$^{loxP/loxP}$; Alb-Cre^{+} (Hep*Pten*$^{-}$). For hepatocyte-specific *Stk3* and *Stk4* deletion, mice carrying *Stk3* and *Stk4* conditional knockout alleles (in a mixed background of CD1, C57BL/6 and 129) were crossed with Albumin (Alb)-Cre-transgenic mice. Control animals were *Stk3*$^{loxP/loxP}$; *Stk4*$^{loxP/loxP}$; Alb-Cre^{-}, whereas the experimental mice were *Stk3*$^{loxP/loxP}$; *Stk4*$^{loxP/loxP}$; Alb-Cre^{+}. At necropsy (approx. 9 months for *Pten* and 4-5 months for *Stk*), tumors and liver tissues were harvested and snap-frozen for further analysis. All animal procedures were carried out in accordance with the policies and regulations set forth by the Institutional Animal Care and Use Committee (IACUC) at MD Anderson Cancer Center.

Hepatocyte-specific myrAKT-N-Ras *overexpression hepatocellular carcinoma model*: Mice were fed standard chow *ad libitum* throughout the procedure.

Orthotopic HUH7 liver cancer model: Mice were fed *ad libitum* a CRM (E) expanded low fat diet (Special Diets Services 801730) one week prior to the start of the experiments until sacrificing. Next, 0.5x10^{6} HUH7 control or shFADS2-2 cells were orthotopically injected in 100% Matrigel^{TM}, (BD Biosciences) into the left liver lobe of anesthetized (3% isoflurane, 2% oxygen) 6-week-old male immunocompromised NMRI$^{nu/nu}$ mice (Taconic M&B AS, Ejby, Denmark). For pain relief, mice were given 5mg per kg carprofen subcutaneous before and after surgery and for the following 3 days. After 2 weeks, mice were euthanized, the tumor nodule was resected, and blood and healthy liver tissue were sampled. For metabolomic analysis, half of the tumor nodule and non-tumor 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 tumor 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 hematoxylin and eosin. The mean tumor area in percent of the total tissue area was determined in low-power magnifications by two independent researcher with pathology training. Housing and experimental animal procedures were approved by the Institutional Animal Care and Research Advisory Committee of the University of Leuven, Belgium.

Collection of clinical samples

Human samples were collected upon ethical approval of local authorities.

Liver: liver and tumor samples were obtained from Indivumed GmbH (Hamburg, Germany) and from the Laboratory of Hepatology (UZ Leuven – KU Leuven, Belgium), respecting patient's rights. Blood samples from healthy volunteers and hepatocellular carcinoma patients were collected in collaboration with the Laboratory of Hepatology (UZ Leuven – KU Leuven, Belgium) 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, Brussels, Belgium. An agreement from the Belgian Ministry of Health was obtained for the Hepatocytes and Hepatic Stem Cells Bank. A written and signed informed consent has been obtained for collection of the cells.

Lung: patients with NSCLC 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. Based on pre-operative imaging and gross inspection at resection, viable fragments of tumor and lung were sampled. Plasma samples from lung cancer patients were drawn primarily from an arterial line throughout the procedure.

Statistical analysis

Statistical data analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., CA, USA) on n≥3 biological replicates. Details on 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 experiments was chosen empirically. Data are presented as mean ± SD, or as mean ± SEM, as indicated in the figure legends.

AUTHOR CONTRIBUTIONS

Investigation and Validation KV, StC, SP, DB, KY, AT, JD, CEN, TC, CC, RS, GR, MR, LRP, MFO, AK, SYK, CCK, LH, TGPG. Formal Analysis KV, StC, TGPG, MFO. Methodology KV, StC, TGPG, SMF. Resource RB, BF, AM, TC, JFG, AS, PS, MN, AH, JAVG, ES, NG, KKW, CaV, RD, SM, MY, LB, RD, JVS, LH, DC, ChV, SvC, SG, TGPG, SMF. Conceptualization KV, StC, SMF. Visualization KV. Writing – Original Draft SMF. Writing – Review & Editing KV, StC, SMF. Supervision SMF. All authors have read and approved the final manuscript. Authors declare there are no competing financial interests.

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DATA AVAILIBILITY

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.

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FIGURE LEGENDS

Figure 1: Cancer cells differentially depend on the stearoyl-CoA desaturase pathway

(**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**) Sensitivity profile of cancer cells treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars; 0.5-2 nM) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Data were normalized to the respective control (DMSO) conditions. Statistically significant differences between control and treated cells were determined via two-way ANOVA followed by Dunnett's multiple comparisons test.

(**C-D**) SCD desaturation activity based on the palmitoleate to palmitate and oleate to stearate ratio in cancer cells. Cells were treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 and T47D at 0.5 nM. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Holm-Sidak multiple comparisons post-hoc test.

(**E**) SCD desaturation activity based on palmitoleate synthesis in cancer cells. Cells were treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars) in low FBS DMEM containing 4.5 g per L U-^{13}C-glucose (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 and T47D at 0.5 nM. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Holm-Sidak multiple comparisons post-hoc test.

(**F**) Palmitate synthesis in cancer cells. Cells were treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars) in low FBS DMEM containing 4.5 g per L U-^{13}C-glucose (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 and T47D at 0.5 nM. Statistically significant differences between control and Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Holm-Sidak multiple comparisons post-hoc test.

(**G**) Correlation analysis between SCD independence upon Merck Frosst Cpd 3j treatment and basal palmitate synthesis. SCD independence is defined as the area under the relative cell number

vs inhibitor dosage curve upon treatment with different concentrations of Merck Frosst Cpd 3j as derived from Figure 1B. Palmitate synthesis data in DMSO condition derived from Figure 1F were used as basal palmitate synthesis abundances. For visualization purposes, linear regression was performed on the dataset, represented by a trend line (dotted line) and 95% confidence intervals (punctuated lines).

(**H**) Correlation analysis between SCD independence upon Merck Frosst Cpd 3j treatment and basal growth rate. SCD independence is defined as the area under the relative cell number *vs* inhibitor dosage curve upon treatment with different concentrations of Merck Frosst Cpd 3j as derived from Figure 1B. For basal growth rate, cells were treated for 72 hours with DMSO in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). For visualization purposes, linear regression was performed on the dataset, represented by a trend line (dotted line) and 95% confidence intervals (punctuated lines).

Data are presented as mean ± SD of n≥3 biological replicates. *, **, *** and **** represent $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively.

Figure 2: Stearoyl-CoA desaturase-independent cancer cells synthesize sapienate

(**A**) Heat map representing the abundances of different fatty acids synthesized by cancer cells. Data values are provided in Extended Data Table **1**. Cells were treated for 72 hours with DMSO or Merck Frosst Cpd 3j in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 and T47D at 0.5 nM. Fatty acid abundances were normalized to the highest abundance of each fatty acid across the panel of cell lines. White indicates a reduction of over 90% in a specific fatty acid compared to the highest abundance of the same fatty acid cross the panel of cell lines. Dark green indicates no reduction in a specific fatty acid compared to the highest abundance of the same fatty acid cross the panel of cell lines.

(**B**) Correlation analysis between SCD independence upon Merck Frosst Cpd 3j treatment and basal desaturation activity of palmitate to sapienate. SCD independence is defined as the area under the relative cell number *vs* inhibitor dosage curve upon treatment with different dosages of Merck Frosst Cpd 3j as depicted in Figure 1B. Data on the DMSO condition derived from Figure 2B were used as basal desaturation activity of palmitate to sapienate. For visualization purposes, linear regression was performed on the dataset, represented by a trend line (dotted line) and 95% confidence intervals (punctuated lines).

(**C**) Desaturation activity to sapienate based on the sapienate to palmitate ratio in cancer cells. Cells were treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 and T47D at 0.5 nM. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Holm-Sidak multiple comparisons post-hoc test.

(**D**) Desaturation activity to sapienate based on sapienate synthesis in cancer cells. BDL refers to below detection limit and indicates that the abundances of sapienate in T47D cells were too low to assess sapienate synthesis. Cells were treated for 72 hours with DMSO (black bars) or Merck Frosst Cpd 3j (white bars) in low FBS DMEM containing 4.5 g per L U-^{13}C-glucose (1% FBS for HUH7; 0.5% FBS for all other cancer cells). Following concentrations of Merck Frosst Cpd 3j were applied: HUH7 and A549 at 2 nM; H460 and DU145 at 1 nM; MDA-MB-468 at 0.5 nM. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Holm-Sidak multiple comparisons post-hoc test.

(**E**) Tumor weight of HUH7 subcutaneous xenografts in mice treated with control (black bars; n=8) and Merck Frosst Cpd 3j (white bars; n=8; 1.5 mg per kg twice daily p.o.). Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-test followed by Welch's correction.

(**F**) Desaturation activity to sapienate based on the sapienate to palmitate ratio in HUH7 xenografts in mice treated with control (black bars; n=8) and Merck Frosst Cpd 3j (white bars; n=8; 1.5 mg per kg twice daily per oral; p.o.). Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-test followed by Welch's correction.

(**G**) Desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in a murine diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) mouse model. Black bars represent healthy liver tissue (n=15); grey bars represent HCC tissue (n=4). Statistically significant differences between healthy liver and tumor tissue were determined by Unpaired Student's T-test followed by Welch's correction.

(**H**) Desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in genetically induced murine HCC models. Black bars represent healthy liver tissue; grey bars represent HCC tissue. Number of replicates were as follows: myrAKT-N-Ras (healthy and HCC

n=10), *Pten* (healthy and HCC n=8) and *Stk* (healthy n=7; HCC n=6). myrAKT-N-Ras refers to myristoylated AKT1 and mutated N-Ras *(*N-Ras^{V12}); PTEN refers to phosphatase and tensin homolog; STK 3,4 refers to serine-threonine kinase 3 and 4. Statistically significant differences between healthy liver and tumor tissue were determined by Unpaired Student's T-test followed by Welch's correction.

(**I**) Desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in HUH7 *versus* freshly isolated primary human hepatocytes (PHH), DU145 *versus* RWPE-1 prostate cells, and MDA-MB-468 and T47D *versus* MCF10A breast cells. HUH7, DU145, MDA-MB-468 and T47D grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). PHH were freshly plated (8 hours) in Corning Plating Medium; RWPE-1 were grown in K-SFM (0.05 mg per mL bovine pituitary extract, 5 ng per mL epidermal growth factor); MCF10A were grown in DMEM-F12 (5% horse serum, 0.5 µg per mL hydrocortisone, 100 ng per mL cholera toxin, 10 µg per mL insulin, 20 ng per mL recombinant human epidermal growth factor). Statistically significant differences between HUH7 and PHH and between DU145 and RWEP-1 cells were determined by Unpaired Student's T-tests followed by Welch's correction. Statistically significant differences between MDA-MB-468, T47D and MCF10A cells were determined by One-Way ANOVA followed by Dunnett's multiple comparisons post-hoc test.

In A, data are presented as mean of n≥3 biological replicates. In B-D and I, data are presented as mean ± SD of n≥3 biological replicates. In E-H, data are presented as mean ± SEM. *, **, *** and **** represent $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively.

Figure 3: Sapienate is synthesized by fatty acid desaturase 2

(**A**) Relative *FADS2* gene expression levels in cancer cells. Cells were grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). *FADS2* expression was normalized to the expression in T47D cells. Statistically significant differences across different cell lines were determined by One-way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**B**) Relative gene expression of *SCD1* and *FADS2* in HUH7-derived xenografts in mice treated with vehicle (black bars; n=7) and Merck Frosst Cpd 3j (white bars; n≥6; 1.5 mg per kg twice daily p.o.). Data were normalized to the vehicle condition. Statistically significant differences between vehicle and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-tests followed by Welch's correction.

(**C**) Correlation analysis between SCD independence upon Merck Frosst Cpd 3j treatment and basal FADS2 protein levels. SCD independence is defined as the area under the relative cell number *vs* inhibitor dosage curve upon treatment with different dosages of Merck Frosst Cpd 3j as depicted in Figure 1B. For visualization purposes, linear regression was performed on the dataset, represented by a trend line (dotted line) and 95% confidence intervals (punctuated lines).

(**D**) Correlation analysis between FADS2 protein levels and basal desaturation activity of palmitate to sapienate. Data on the DMSO condition derived from Figure 2B were used as basal desaturation activity of palmitate to sapienate. For visualization purposes, linear regression was performed on the dataset, represented by a trend line (dotted line) and 95% confidence intervals (punctuated lines).

(**E**) Relative FADS2 protein expression levels in HUH7 *versus* freshly isolated primary human hepatocytes (PHH), DU145 *versus* RWPE-1 prostate cells, and MDA-MB-468 and T47D *versus* MCF10A breast cells. HUH7, DU145, MDA-MB-468 and T47D grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cancer cells). PHH were freshly plated (8 hours) in Corning Plating Medium; RWPE-1 were grown in K-SFM (0.05 mg per mL bovine pituitary extract, 5 ng per mL epidermal growth factor); MCF10A were grown in DMEM-F12 (5% horse serum, 0.5 µg per mL hydrocortisone, 100 ng per mL cholera toxin, 10 µg per mL insulin, 20 ng per mL recombinant human epidermal growth factor). Statistically significant differences between HUH7 and PHH and between DU145 and RWEP-1 cells were determined by Unpaired Student's T-tests followed by Welch's correction. Statistically significant differences between MDA-MB-468, T47D and MCF10A cells were determined by One-Way ANOVA followed by Dunnett's multiple comparisons post-hoc test.

(**F, G**) *FADS2* gene expression in paired human hepatocellular carcinoma *versus* healthy adjacent liver tissue (n=4) and paired human lung cancer versus healthy adjacent lung tissue (n=XXX).

(**H**) Desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in HUH7 and A549 cells with a non-targeting (NT) shRNA (control; black bars) or two different shRNA targeting FADS2 (brown and orange bars). Cells were grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to the respective control conditions. Statistically significant differences between control and shFADS2 cells were determined by One-Way ANOVA followed by Dunnett's multiple comparison post-hoc test.

(**I**) Desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in healthy liver (n=5) and HUH7 orthotopic liver tumors (n=5). Injected HUH7 cells carried either a

non-targeting shRNA (control; black bars) or a shRNA targeting FADS2 (orange bars). Statistically significant differences between healthy liver tissue and liver tumors within control or FADS2 knockdown condition were determined by Two-Way Repeated Measures ANOVA, followed by Sidak's multiple comparison post-hoc test. Data are presented as mean ± SEM.

Data are presented as mean ± SD of n≥3 biological replicates, unless stated otherwise. *, **, *** and **** represents $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively.

Figure 4: Sapienate biosynthesis causes stearoyl-CoA desaturase-independence

(**A**) Relative desaturation activity from palmitate to sapienate based on the sapienate to palmitate ratio in MDA-MB-468 cells with empty pLVX (control; black bars) or pLVX-FADS2 (green bars) for overexpression of FADS2. Cells were grown in 0.5% FBS DMEM. Data were normalized to control condition. Statistically significant differences between control and FADS2 overexpression were determined by Unpaired Student's T-test.

(**B**) Relative proliferation of MDA-MB-468 control (empty pLVX; black bars) and pLVX-FADS2 (green bars) cells upon 72 hours treatment with DMSO (dark bars) or 0.5 nM Merck Frosst Cpd 3j (light bars) in 0.5% FBS DMEM. Relative proliferation was assessed based on cell number. Data were normalized to control condition. Statistically significant differences between DMSO and Cpd 3j treatment were determined by Two-Way ANOVA followed by Holm-Sidak multiple comparisons post-hoc test.

(**C**, **D**) Relative proliferation of HUH7 and A549 control (non-targeting shRNA; black and white bars) and knockdown (shFADS2; brown and orange bars) cells with ethanol, 20 µM palmitoleate, or 20 µM sapienate upon 72 hours treatment with DMSO (dark bars) or 2 nM Merck Frosst Cpd 3j (light bars) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Relative proliferation was assessed based on cell number. Data were normalized to control+DMSO condition. Statistically significant differences between control and Cpd 3j treatment were determined by Two-Way ANOVA followed by Tukey multiple comparisons post-hoc test. For visibility, only statistical significant differences between DMSO and SCD inhibition in each condition (pairwise) are presented.

(**E**) Representative images of hematoxylin and eosin stain on tumor nodules derived from HUH7 control (non-targeting shRNA) or FADS2 knockdown (shFADS2) orthotopic xenografts in mice

treated with control or Merck Frosst Cpd 3j (1.5 mg per kg twice daily per oral; p.o.). Tumor areas are circled in green. L, T and N refer to liver, tumor and necrotic area, respectively.

(**F**) Relative tumor area in nodules derived from HUH7 control (non-targeting shRNA) or FADS2 knockdown (shFADS2) orthotopic xenografts in mice treated with control or Merck Frosst Cpd 3j (1.5 mg per kg twice daily per oral; p.o.). Relative tumor area is defined as tumor area to nodule area ratio, and was assessed via hematoxylin and eosin stain. Data of n≥13 are presented as box plots with whiskers indicating the minimum and maximum and a line indicating the mean. Statistically significant differences between groups were determined by One-Way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**G**) Relative desaturation activity from linoleate to γ-linolenate based on the γ-linolenate to linoleate ratio in HUH7 and A549 control (non-targeting shRNA; black bars) and FADS2 knockdown (shFADS2; brown and orange bars) cells. Cells were grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to control condition. Statistically significant differences between control and FADS2 knockdown cells were determined by One-Way ANOVA followed by Dunnett's post hoc test.

(**H**) Relative arachidonate abundance in HUH7 and A549 control (non-targeting shRNA; black bars) and FADS2 knockdown (shFADS2; brown and orange bars) cells. Cells were allowed to attach for 24 hours and subsequently grown for 72 hours in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to control condition. Statistically significant differences between control and FADS2 knockdown cells were determined by One-Way ANOVA followed by Dunnett's post hoc test.

(**I**) Relative linoleate abundance in healthy liver and tumor nodules derived from HUH7 control (non-targeting shRNA) or FADS2 knockdown (shFADS2) orthotopic xenografts in mice treated with control or Merck Frosst Cpd 3j (1.5 mg per kg twice daily per oral; p.o.). Data were normalized to healthy liver of control mice and presented as mean ± SEM of n≥9 biological replicates. Statistically significant differences between groups were determined by Two-Way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**J**) Relative arachidonate abundance in healthy liver and tumor nodules derived from HUH7 control (non-targeting shRNA) or FADS2 knockdown (shFADS2) orthotopic xenografts in mice treated with control or Merck Frosst Cpd 3j (1.5 mg per kg twice daily per oral; p.o.). Data were normalized to healthy liver of control mice and presented as mean ± SEM of n≥13 biological

replicates. Statistically significant differences between groups were determined by Two-Way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**K**) Relative proliferation of MDA-MB-468 cells with ethanol, 20 µM palmitoleate or 20 µM sapienate upon 72 hours treatment with DMSO (black bars) or 0.5 nM Merck Frosst Cpd 3j (white bars) in 0.5% FBS DMEM. Relative proliferation was assessed based on cell number. Data were normalized to control condition. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Two-Way ANOVA followed by Tukey multiple comparisons post-hoc test. For visibility, only statistically significant differences between DMSO and SCD inhibition in each condition (pairwise) are presented.

Data are presented as mean ± SD of n≥3 biological replicates, unless stated otherwise. *, **, *** and **** represents $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively; § represents non-significant differences.

Figure 5: Sapienate is elongated and used for membrane synthesis

(**A**) Relative cis-8-octadecenoate abundances in the cancer cell line panel used in this study. Cells were grown in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for all other cells). Abundances were normalized to those of T47D cells. Statistically significant differences between cells were determined by One-Way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**B**, **C**) Relative cis-8-octadecenoate abundances in HUH7 and A549 control (non-targeting shRNA; black bars) and FADS2 knockdown (shFADS2; brown and orange bars) cells in control condition (ethanol) or upon 20 µM ^{12}C sapienate supplementation for 72 hours in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to control condition. Data values are depicted in Extended Data Table **3** and **4**. Statistically significant differences between the different conditions were determined by Two-Way ANOVA followed by Tukey's multiple comparisons post-hoc test.

(**D**, **E**) Relative palmitoleate, sapienate, oleate and cis-8-octadecenoate abundances in HUH7 and A549 cells treated with control or 2 nM Merck Frosst Cpd 3j for 72 hours in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to the respective control conditions. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-test.

(**F**) Relative proliferation of MDA-MB-468 cells with ethanol or 20 µM cis-8-octadecenoate upon 72 hours treatment with DMSO (black bars) or 0.5 nM Merck Frosst Cpd 3j (white bars) in 0.5% FBS DMEM. Relative proliferation was assessed based on cell number. Data were normalized to the respective control condition and are presented as mean ± SEM of n≥3. Statistically significant differences between the different conditions were determined by Two-Way ANOVA followed by Tukey multiple comparisons post-hoc test.

(**G**, **H**) Relative proliferation of HUH7 and A549 control (non-targeting shRNA; black bars) and knockdown (shFADS2; brown and orange bars) cells with ethanol or 20 µM cis-8-octadecenoate upon 72 hours treatment with DMSO (dark bars) or 2 nM Merck Frosst Cpd 3j (light bars) in low FBS DMEM (1% FBS for HUH7; 0.5% FBS for A549). Relative proliferation was assessed based on cell number. Data were normalized to the control+DMSO condition. Statistically significant differences between the different conditions were determined by Two-Way ANOVA followed by Tukey multiple comparisons post-hoc test. For visibility, only statistically significant differences between DMSO and SCD inhibition in each condition (pairwise) are presented.

(**I**) ^{13}C enrichment of octadecenoate from $^{13}C_6$ glucose in HUH7 cells in control condition (ethanol, black bars) or upon 20 µM ^{12}C sapienate supplementation (blue bars). Cells were grown in 1% FBS DMEM containing 4.5 g per L $^{13}C_6$ glucose for 1 week, after which cells were grown for 72 hours in 1% FBS DMEM containing 4.5 g per L $^{13}C_6$ glucose supplemented with ethanol or 20 µM ^{12}C sapienate. Statistically significant differences between control and sapienate treatment were determined via Unpaired Student's T-tests.

(**J**) ^{13}C enrichment of octadecenoate from $^{13}C_6$ glucose in A549 cells in control condition (ethanol, black bars) or upon 20 µM ^{12}C sapienate supplementation (blue bars). Cells were grown in 10% dialyzed FBS DMEM containing 4.5 g per L $^{13}C_6$ glucose for 1 week, after which cells were grown for 72 hours in 0.5% FBS DMEM containing 4.5 g per L $^{13}C_6$ glucose supplemented with ethanol or 20 µM ^{12}C sapienate. Statistically significant differences between control and sapienate treatment were determined via Unpaired Student's T-tests.

(**K**) Palmitoleate and sapienate abundances in membrane phospholipids in HUH7 cells carrying a non-targeting shRNA (black bars) and a shRNA targeting FADS2 (orange bars). Cells were grown in 1% FBS DMEM. Statistically significant differences between control and FADS2 knockdown cells were determined by Unpaired Student's T-test.

(**L**-**M**) Relative phospholipid-bound palmitoleate, sapienate and cis-8-octadecenoate abundances in HUH7 and A549 cells treated with control or 2 nM Merck Frosst Cpd 3j for 72 hours in low FBS

DMEM (1% FBS for HUH7; 0.5% FBS for A549). Data were normalized to the respective control conditions. BDL refers to below detection limit. When the respective control was BDL, the data were normalized to the total abundance of all fatty acids measured and is represented in arbitrary units. Statistically significant differences between control and Merck Frosst Cpd 3j treatment were determined by Unpaired Student's T-test.

Data are presented as mean ± SD of n≥3 biological replicates. For all graphs, *, **, *** and **** represent *P*<0.05, *P*<0.01, *P*<0.001 and *P*<0.0001, respectively.

Figure 6: Sapienate synthesis occurs in human lung and liver cancers

(**A**) Relative sapienate to palmitate and palmitoleate to palmitate ratios in hepatocellular carcinoma and healthy liver tissue as well as blood plasma from humans. Healthy blood plasma was from volunteers, while healthy liver was adjacent non-cancerous tissue from cancer patients and non-transplanted donor livers (healthy: blood plasma n=23, tissue n=16 and cancer: blood plasma n=33, tissue n=16). Black indicates blood plasma and grey indicates tissue. Notably, blood plasma ratios from healthy volunteers are the same as in Figure 6B. Data were normalized to healthy liver tissue.

(**B**) Relative sapienate to palmitate and palmitoleate to palmitate ratios in lung cancer and healthy lung tissue as well as blood plasma from humans. Healthy blood plasma was from volunteers, while healthy lung was adjacent non-cancerous tissue from cancer patients (Healthy: blood plasma n=23, tissue n=15 and cancer: blood plasma: n=34, tissue n=15). Black indicates blood plasma and grey indicates tissue. Notably, blood plasma ratios from healthy volunteers are the same as in Figure 6A. Data were normalized to healthy lung tissue.

Statistically significant differences between the different conditions were determined by Two-Way ANOVA followed by Tukey multiple comparisons post-hoc test. Data are presented as mean ± SEM of n≥15 biological replicates. *, **, *** and **** represent *P*<0.05, *P*<0.01, *P*<0.001 and *P*<0.0001, respectively.