Supplementary Note 1; Insulin did not increase proliferation rates of tested cell lines
Consistent with the absence of hepatic insulin resistance, no difference was observed in the rate of gluconeogenesis between mice on a control diet with or without 10% fructose water supplementation for 6 weeks (Extended Data Fig. 1i). Furthermore, the four cell lines in which HFCS stimulated growth did not proliferate faster with insulin administration in media enriched with 0.1% fetal bovine serum (FBS), indicating that insulin is not mitogenic in these models (Extended Data Fig. 1j-m).

Supplementary Note 2; Serum fructose after large bolus
Under fasting conditions, the level of fructose in the serum is low or undetectable. Fructose levels spike in serum following ingestion of fructose, typically staying below millimolar concentrations even at peak levels and before returning to baseline within less than a few hours[1–4]. After administering a large bolus (1g/kg) of fructose to mice via intraperitoneal (IP) injection, we observed that serum fructose peaked at approximately 1.3 mM and was mostly cleared by 2 hours (Extended Data Fig. 2a). IP administration increased serum bioavailability of fructose over oral ingestion, where only some fructose makes it past the intestine and liver[1,4]

Supplementary Note 3; Cancer cells perform fructoneogenesis
Cells within an organism potentially have access to exogenous and endogenous fructose. Exogenous fructose comes from the diet. Endogenous fructose is produced in cells that express the enzymes aldose reductase and sorbitol dehydrogenase. Through the polyol pathway, glucose can be transformed into sorbitol by aldose reductase, which can then form fructose by sorbitol dehydrogenase (Extended Data Fig. 5a). We discovered that a variety of cancers express sorbitol dehydrogenase and thus have the capacity for fructoneogenesis (Extended Data Fig. 5b). After labelling with [U-^{13}C] glucose for 4 hours and applying a liquid chromatography method that could resolve fructose and glucose, we found evidence of fructose production in all cancer cell lines tested (Fig. 1l; Extended Data Fig. 5c-5i).

To understand whether the endogenous fructose being produced via sorbitol dehydrogenase contributes to cancer cell proliferation, we silenced sorbitol dehydrogenase in multiple cell lines cultured in dialyzed FBS containing no exogenous fructose (Extended Data Fig. 6a). Under these conditions, we observed either a decrease or no change in cell proliferation (Fig 1m; Extended Data Fig. 6b-f). For cells in which sorbitol dehydrogenase silencing reduced proliferation, adding fructose back to the medium did not provide a rescue (Fig 1m; Extended Data Fig. 6b-f). These data suggest that the fructose produced de novo by the polyol pathway is not important for cancer cell proliferation in our models. More investigation is needed to understand the role of fructoneogenesis in these cells, but it might be related to NAD^+/NADH levels[5,6].

Supplementary Note 4; Fructose is poorly metabolized by hexokinase
Having determined that fructose carbons do not enter central carbon metabolism via KHK in CaSki cells, we next assessed the contribution of hexokinase to fructose metabolism. While understanding the role of KHK in tumours remains an active area of investigation, the role of hexokinase in cancer cell metabolism is well established[7–9]. Co-administration of 20 mM 2-deoxyglucose with [U-^{13}C] fructose significantly reduced M+3 lactate labelling in CaSki cells, indicating that the minimal amount of fructose metabolism observed in cells is a result of hexokinase rather than KHK, which is consistent with a previous report[10] (Extended Data Fig. 7b). The observation that fructose 6-phosphate is labelled much less from [U-^{13}C] fructose

relative to [U-^{13}C] glucose indicates that hexokinase has a decreased capacity to use fructose as a substrate compared to glucose (Extended Data Fig. 7c). These results are consistent with previous reports that hexokinase has a significantly higher k_m for fructose relative to glucose[11–14]. On the basis of our data, we conclude that hexokinase cannot efficiently utilize fructose as a substrate in cancer cells.

Supplementary Note 5; FBS contains millimolar levels of fructose

Although we deemed it plausible that expression of KHK-C and aldolase-B might be reduced when cells are consistently cultured without fructose, we found that the FBS used in these experiments contained millimolar levels of fructose (Extended Data Fig. 7d). This result is consistent with reports that ruminants *in utero* have high blood fructose[15,16]. Thus, cells grown in culture with 10% FBS are exposed to levels of fructose that are in the upper range of postprandial fructose concentrations in mouse and human serum, but this is not sufficient to drive expression of fructolytic enzymes

Supplementary Note 6; Cancer cells isolated from tumours do not metabolize fructose efficiently

We wanted to confirm that cancer cells isolated from tumours were similarly deficient in their capacity to metabolize fructose. To this end, we resected CaSki tumours from nude mice 3 weeks after transplantation. While the tumours were growing, mice were maintained on a control diet with 10% fructose water supplementation. After resection of the tumour, a single-cell suspension was formed and the cells were plated in culture for 5 hours to allow cell attachment to culture plates. During the attachment process, we used media containing 5 mM glucose and 5 mM fructose as the only simple sugars to allow for continued exposure to fructose. We then cultured the cells in 1:1 [U-^{13}C] fructose: glucose or 1:1 [U-^{13}C] glucose: fructose for 4 hours prior to metabolomics analysis. We observed extensive metabolism of the glucose label, but only minimal metabolism of the fructose label (Extended Data Fig. 7g).

Supplementary Note 7; Translation to human cervical cancer patients

To examine the translational relevance of our findings that KHK-A and KHK-C activity are not contributing to the growth of cervical tumours, we used whole transcriptome sequencing (RNA-seq) data to measure *KHK-A* and *KHK-C* isoform expression in primary cervical cancer samples from a cohort of 99 patients uniformly treated with chemoradiation at Washington University School of Medicine. Overall, we observed that the relative expression of *KHK-A* and *KHK-C* from all of the human cervical tumours was consistent with our *in vitro* data. None of the cervical tumours showed *KHK-C* expression, while all of the cervical tumours expressed low levels of *KHK-A* with a mean of 0.876 FPKM (Fig. 2e). This is also confirmed by examining read coverage of exons that are specific to *KHK-A* (Exon 3A) or *KHK-C* (Exon 3C), and splice junction reads at these exons (Extended Data Fig. 7k). The function of KHK-A in cancer is poorly understood, with some studies suggesting a role for KHK-A in neoplastic cells that is potentially independent of fructose[17–19]. Our cell-culture data indicate that KHK-A activity is not important for cervical cancer cell proliferation because inhibition of KHK-A with PF-06835919 did not impact cell number (Extended Data Fig. 7h). Consistent with this finding, we also determined that *KHK-A* expression was not significantly associated with cervical cancer stage or overall survival in this cohort of human patients (Fig. 2f-g). Furthermore, we determined that aldolase B was not expressed in the primary cervical tumour samples of this same patient cohort

(Fig. 2h). Therefore, these human cervical tumours do not express either of the fructolytic enzymes KHK-C and aldolase B.

Supplementary Note 8; Glucose and aspartate are fructose-derived, polar metabolites released by hepatocytes and consumed by cancer cells

In a second and independent analysis, we performed the same screen for polar metabolites as we did for lipid metabolites (Extended Data Fig. 8a). Profiling of over 100 polar metabolites in hepatocyte CM revealed a set of metabolites that were ^{13}C-enriched from [U-^{13}C] fructose (Extended Data Fig. 8b). Of all the ^{13}C-enriched metabolites in hepatocyte CM, only glucose and aspartate had statistically significant pool-size decreases in CaSki CM (Extended Data Fig. 8c). Other ^{13}C-labelled metabolites excreted by hepatocytes were either unchanged or also excreted by CaSki cells (Extended Data Fig. 8c).

Supplementary Note 9; Zebrafish have a KHK isoform distribution similar to mammals

A study of KHK isoforms in zebrafish has yet to be been reported. We aimed to determine whether zebrafish express KHK isoforms analogous to KHK-A and KHK-C in humans. To compare the expression of different KHK isoforms in zebrafish to KHK isoforms in humans, we used EMBOSS Needle[20] to pairwise align human and zebrafish *KHK* DNA sequences. According to the alignment, zebrafish *khk-201* is analogous to *KHK-C* in humans, while *khk-202* in zebrafish is analogous to *KHK-A* in humans (Extended Data Fig. 9a). The analysis shows that Exon 3 in *khk-201* corresponds to Exon 3 in *KHK-C*, while Exon 3 in *khk-202* corresponds to Exon 3 in *KHK-A*. We then used RNA-seq data to investigate the expression of different *KHK* isoforms in thirteen zebrafish tissues, including the blood, brain, colon, embryonic brain, embryonic trunk, heart, intestine, kidney, liver, muscle, skin, spleen, and testis (each tissue was replicated twice). Overall, we observed *KHK* expression (>1 FPKM) in all 12 tissue types (ranges in 1.0 to 131.7 FPKM), while there was no *KHK* expression in the blood. Among these 12 tissues with *KHK* expression, 4 tissue types (colon, intestine, kidney and liver) have the highest *KHK* expression, specifically stemming from the *khk-201* isoform (FPKM values are 57.3, 123.8, 52.8, 51.1, respectively) (Extended Data Fig. 9b). We confirmed this pattern by examining exon-specific expression and splicing reads by using IGV[21]. These findings demonstrate that khk-201 tissue expression correlates with KHK-C expression in humans (where KHK-C is highly expressed in the liver, kidney, and small intestine). Protein analysis also revealed expression of aldolase B, which is required to transform fructose 1-phosphate into glycolytic intermediates, in the livers of zebrafish (Extended Data Fig. 9c). Additionally, administration of PF-06835919 to zebrafish had effects on fructose levels and isotope labelling patterns that are consistent with KHK inhibition (Extended Data Fig. 9d-e) and rescued fructose-mediated regrowth of tumours (Extended Data Fig. 9f).

Zebrafish are omnivores, consuming a variety of animal and plant matter. Fructans, a type of soluble fiber, are polymers of fructose that occur in many plants, algae, and bacteria[22]. Although zebrafish diets are unlikely to be comprised of fructose itself, we imagined that the breakdown of fructans by zebrafish microbiota might result in fructose monomers whose metabolism requires *khk-201* activity (Extended Data Fig. 9b). To test this possibility, we orally gavaged zebrafish with 1.5 g/kg [U-^{13}C] inulin. Subsequent LC/MS analysis revealed [U-^{13}C] fructose monomers in the intestines and serum of zebrafish (Extended Data Fig. 9g). Removal of the microbiota with

antibiotic treatment substantially reduced the production of [U-^{13}C] fructose (Extended Data Fig. 9g). Thus, we conclude that zebrafish can utilize *khk-201* to degrade fructan-derived fructose

Supplementary Note 10: Hepatic fructose metabolism increases de novo lipogenesis to promote tumour growth

We have demonstrated that dietary fructose promotes the growth of multiple types of tumours. Our data indicate that the process is cell non-autonomous in that tumours do not readily metabolize fructose directly, but rather consume hepatic LPCs that are made from fructose. It is important to point out, however, that LPCs derived from fructose are not unique. A complementary cancer-cell extrinsic mechanism for dietary fructose to promote tumour growth is for dietary fructose to induce hepatic synthesis of lipids from non-fructose substrates, such as glucose. It is already established that the product of KHK-C, fructose 1-phosphate, activates hepatic glucokinase and increases de novo lipogenesis[23–25]. Consistent with prior evidence, we found that six weeks of high-fructose consumption resulted in greater hepatic expression of acetyl-CoA carboxylase, which catalyzes the rate-limiting enzyme of de novo lipogenesis (Extended Data Fig. 10d). Additionally, daily administration of PF-06835919 via IP injection rescued the increased expression of hepatic acetyl-CoA carboxylase in mice supplemented with 10% fructose water for 5 days (Extended Data Fig. 10e, f).

To assess de novo lipogenesis directly, we administered D_2O to mice and measured deuterium enrichment in hepatic lipids. Specifically, we provided mice with access to normal water, 10% fructose water, or 10% fructose water with daily IP injection of 50 mg/kg PF-06835919 for seven days. We then provided 50% D_2O in the drinking water while maintaining mice under the same experimental conditions for 12 hours. Analysis of mouse livers by LC/MS showed that fructose supplementation enhances the relative deuterium enrichment in hepatic free fatty acids, and this enhancement is rescued by administration of PF-06835919 (Extended Data Fig. 10g-i). The results reveal that the transformation of fructose to fructose 1-phosphate by KHK in fructolytic tissues drives increased lipid synthesis, irrespective of which substrate is used to produce acetyl-CoA.

Supplementary Note 11: Fructose carbons contribute to the glycerol headgroup and fatty acyl chain of LPCs

Fructose can contribute carbons to either the glycerol headgroup or fatty acyl chain of an LPC via glycerogenesis or de novo lipogenesis, respectively (Figure 2a). To estimate the contribution that fructose makes to each molecular component of LPCs, we used fractional carbon atom labelling [26]. The amount of glycerol carbon in circulating LPCs that is derived from fructose was evaluated on the basis of LPC 18:2 in serum (Extended Data Fig. 11c, d). Given that LPC 18:2 has an essential acyl chain, only the glycerol portion of the LPC can be labelled. After 84 hours of ad lib access to 10% [U-^{13}C] fructose water, we concluded that at least 40% of glycerol carbon in the fed state and at least 24% of glycerol carbon in the fasted state are derived from fructose (Extended Data Fig. 11d). To assess the amount of fatty acid in circulating LPCs that is derived from fructose, we analyzed 16:0 acylcarnitine in the same animals. Acylcarnitine was used instead of free fatty acid to avoid background palmitate signal[27]. It was determined that at least 10% of 16:0 carbons in the fed state and at least 13% of 16:0 carbons in the fasted state are derived from fructose (Extended Data Fig. 11d). We note that the quantitative contributions of fructose reported here are only lower estimates because the experiments were not performed at isotopic steady state.

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