Breast cancer cells rely on environmental pyruvate to shape the metastatic niche

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The extracellular matrix is a major component of the local environment—that is, the niche—that determines cell behaviour1. During metastatic growth, cancer cells shape the extracellular matrix of the metastatic niche by hydroxylating collagen to promote their own metastatic growth2,3. However, only particular nutrients might support the ability of cancer cells to hydroxylate collagen, because nutrients dictate which enzymatic reactions are active in cancer cells4,5. Here we show that breast cancer cells rely on the nutrient pyruvate to drive collagen-based remodelling of the extracellular matrix in the lung metastatic niche. Specifically, we discovered that pyruvate uptake induces the production of α-ketoglutarate. This metabolite in turn activates collagen hydroxylation by increasing the activity of the enzyme collagen prolyl-4-hydroxylase (P4HA). Inhibition of pyruvate metabolism was sufficient to impair collagen hydroxylation and consequently the growth of breast-cancer-derived lung metastases in different mouse models. In summary, we provide a mechanistic understanding of the link between collagen remodelling and the nutrient environment in the metastatic niche.

The metabolic requirements of extracellular matrix (ECM) production and modification can be enforced in vitro by shifting the growth of cancer cells from an attached monolayer (two-dimensional) to a spheroid (three-dimensional). In the latter condition, a soft-agar coating prevents cells from attachment and thus induces the need to generate ECM in cells to enable effective growth. We postulated that nutrients that drive three-dimensional (but not two-dimensional) growth could be a requirement for ECM metabolism. Consequently, we depleted glucose, glutamine or pyruvate from the medium and assessed the growth of MCF10A cells in which H-RAS was mutated (H-RASV12) and 4T1 cells in three-dimensional compared to two-dimensional cultures. Pyruvate has previously been identified to be particularly available in the lung6,7, which is a frequent metastatic site of breast cancers. We found that only depletion of pyruvate impaired the three-dimensional growth of breast cancer cells, while having only a minor effect on two-dimensional growth (Fig. 1a and Extended Data Fig. 1a, b). This identifies pyruvate as a nutrient that is potentially important for ECM metabolism. In this case, we expect that ECM supplementation (Matrigel) restores three-dimensional growth in the absence of pyruvate.

Fig. 1 | Pyruvate drives ECM remodelling via collagen hydroxylation. a, Growth response of MCF10A H-RASV12 two-dimensional and three-dimensional cultures with or without glucose (17.5 mM), glutamine (2.5 mM) or pyruvate (0.5 mM). Growth was assessed based on cell number (two-dimensional, n = 6) or spheroid size (three-dimensional, n = 3). b, Representative pictures of MCF10A H-RASV12 spheroids with or without pyruvate and supplemented with ECM (Matrigel). Analysis was performed at day 5. Scale bar: 150 μm. c, Relative change in pyruvate uptake in MCF10A H-RASV12 spheroids with or without supplemented ECM (Matrigel) normalized to the condition with pyruvate, n = 6. d, Hydroxylated collagen based on hydroxyproline (OH-proline) in human (MCF10A, MCF10A H-RASV12, MCF7, HCC70) and mouse (4T1, EMT6.5) breast cancer spheroids with or without pyruvate, n = 3 for MCF10A and EMT6.5, n = 6 for MCF7 and HCC70; n = 9 for MCF10A H-RASV12 and 4T1. e, Hydroxylated collagen based on hydroxyproline content in breast cancer spheroids transduced with lentiviral CRISPR with or without guide RNA (gRNA) for human MCT2 (or mouse Mct2) in the presence of pyruvate, n = 6 for control gRNA; n = 3 for MCT2 (or Mct2) gRNA 1 and 2. f, Collagen stability based on the hydroxyproline distribution between MCF10A H-RASV12 cells and supernatant upon MMP8 digestion with or without pyruvate or cell-permeable α-ketoglutarate (dimethyl 2-oxoglutarate; α-KG; 1.5 mM), n = 3. Error bars represent s.d. of mean from biological independent samples. Two-tailed unequal Student’s t-test.

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Pyruvate and decreases pyruvate uptake. Indeed, Matrigel induced the expected alterations in MCF10A H-RASV12 cells (Fig. 1b, c). Notably, non-tumorigenic MCF10A cells were pyruvate independent (Extended Data Fig. 1c). Thus, we concluded that pyruvate supports ECM metabolism in breast cancer cells.

Next, we investigated the influence of pyruvate on collagen-based ECM production and modification by cancer cells (Extended Data Fig. 1d). We used different human (MCF10A H-RASV12, MCF7 and HCC70) and mouse (4T1, EMT6.5) breast cancer cells and assessed collagen hydroxylation (ECM modification) and collagen synthesis (ECM production). Non-tumorigenic MCF10A cells were used as control. We found that pyruvate significantly increased hydroxylation of collagen in all cancer cells (Fig. 1d), but had no effect on non-tumorigenic MCF10A cells and collagen synthesis (Fig. 1d and Extended Data Fig. 2a, b). We obtained similar results by targeting pyruvate uptake (by inhibiting the pyruvate transporter monocarboxylate transporter 2; MCT2) and pyruvate metabolism (by inhibiting the mitochondrial pyruvate carrier) (Fig. 1e and Extended Data Fig. 3a–c). These results suggest that pyruvate is required for collagen modification (that is, hydroxylation) rather than synthesis.

Because hydroxylation is essential for collagen stability, we next measured the stability of collagen produced by MCF10A H-RASV12 and 4T1 cells using a matrix metalloproteinase 8 (MMP8) assay. MMP8 digests collagen I–III, but digestion is impaired by increased stability. If pyruvate drives collagen stability through hydroxylation, we expect that upon pyruvate depletion, MMP8 is more effective in digesting collagen that is produced by cancer cells. Specifically, we measured the hydroxyproline distribution between cells and supernatant because only hydroxyproline from digested collagen is released into the supernatant. We observed that pyruvate depletion significantly decreased the stability of collagen produced by MCF10A H-RASV12 and 4T1 cells (Fig. 1f). Thus, we concluded that pyruvate drives ECM remodelling by inducing collagen hydroxylation, which results in increased collagen stability.

We next investigated the mechanism by which pyruvate drives collagen hydroxylation. We hypothesized that metabolites that changed pyruvate and decreases pyruvate uptake. Indeed, Matrigel induced the expected alterations in MCF10A H-RASV12 cells (Fig. 1b, c). Notably, non-tumorigenic MCF10A cells were pyruvate independent (Extended Data Fig. 1c). Thus, we concluded that pyruvate supports ECM metabolism in breast cancer cells.

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Fig. 4 | Pyruvate drives in vivo collagen hydroxylation and metastatic growth. a. Metabolite abundances in 4T1 mice upon MCT2 inhibition (α-cyano-4-hydroxycinnamic acid; 60 mg per kg; i.p.). Plasma n = 10 exception pyruvate with MCT2 inhibitor n = 9; tissue pyruvate/lactate n = 5 and α-ketoglutarate n = 10. b. Hydroxylated collagen in 4T1 and EMT6.5 lung metastases upon pharmacological (α-cyano-4-hydroxycinnamic acid; 60 mg per kg; i.p.) and genetic Mct2 inhibition (n = 5). c. Functional collagen in the same models as described in b. Significance collagen red/green reduction: 0.009/0.006 (4T1 inhibitor), 0.01/0.006 (4T1 genetic) and 0.001/0.04 (EMT6.5 genetic). n = 10 (4T1 vehicle), n = 7 (4T1 inhibitor), n = 11 (4T1 control), n = 6 (4T1 Mct2 gRNA), n = 20 (EMT6.5 control), n = 12 (EMT6.5 Mct2 gRNA). d. Hydroxylated collagen in 4T1 lung metastases upon MCT2 inhibition (α-cyano-4-hydroxycinnamic acid; 60 mg per kg; i.p.) with(out) cell-permeable α-ketoglutarate (50 mg per kg; i.p.; n = 5). e. Functional collagen in the same models as described in d. Significance collagen red/green increase: 0.0008/0.0005. n = 7 (inhibitor), n = 9 (inhibitor + α-ketoglutarate). f. Hydroxylated collagen in 4T1 lung metastases upon genetic inhibition of Alt2 (also known as Gpt2) (n = 5). g. Functional collagen in the same models as described in f. Significance collagen red/green reduction: 0.10/0.02. n = 4 (Alt2 shRNA). h, i. Metastatic burden in 4T1 and EMT6.5 lungs upon genetic Mct2 or Alt2 inhibition. n = 11 (4T1 control; two cohorts), n = 7 (4T1 Mct2 gRNA; two cohorts), n = 10 (EMT6.5 control), n = 10 (EMT6.5 Mct2 gRNA or shAlt2). j. Metastatic burden in the same models as described in d. n = 23 (vehicle; three cohorts), n = 24 (inhibitor; two cohorts), n = 10 (inhibitor + α-ketoglutarate). k. Role of pyruvate in ECM remodelling, m.f.: mammary fat pad; i.v.: intravenous; i.p.: intraperitoneal. Dashed lines indicate level without treatment. Data are normalized to no treatment condition. Error bars represent s.e.m. from different mice. Two-tailed unpaired Student’s t-test.

In abundance upon pyruvate depletion could mechanistically link this nutrient to collagen hydroxylation. We found that upon pyruvate depletion the abundance of α-ketoglutarate, citrate and malate were reduced in MCF10A H-RASV12 spheroids (Extended Data Fig. 4a, b). Lactate, a metabolite that can be taken up by cancer cells and converted into pyruvate, was not altered in abundance and did not influence collagen hydroxylation (Extended Data Fig. 4a, c). Consequently, we measured hydroxylated collagen in the presence of α-ketoglutarate, citrate and malate and observed that only addition of α-ketoglutarate significantly increased the levels of hydroxylated collagen (Fig. 2a and Extended Data Fig. 4d). This finding was confirmed in multiple cell lines and upon inhibition of human MCT2 (also known as SLC16A7) or mouse Mct2 (also known as Slc16a7) (Fig. 2b and Extended Data Fig. 4e, f). Moreover, addition of α-ketoglutarate rescued collagen stability upon...
pyruvate depletion (Fig. 1f). Thus, we concluded that pyruvate-induced production of \(\alpha\)-ketoglutarate drives collagen hydroxylation.

Pyruvate metabolism can be linked to \(\alpha\)-ketoglutarate via several metabolic pathways. The most direct metabolic link is the alanine aminotransferase (ALT) reaction that converts pyruvate and glutamate to \(\alpha\)-ketoglutarate and alanine (Extended Data Fig. 1d). Measuring the \(^{13}\)C distribution in the metabolites of the ALT reaction, we observed that pyruvate contributed more carbon to alanine than to \(\alpha\)-ketoglutarate and that glutamine (an important glutamate precursor) donated about 45% of carbon to \(\alpha\)-ketoglutarate (Extended Data Fig. 5a). Moreover, alanine was secreted specifically in the presence of pyruvate (Extended Data Fig. 5b) and addition of neither alanine nor glutamate rescued hydroxylated collagen abundance upon pyruvate depletion (Extended Data Fig. 4c). These findings indicate an involvement of ALT in the pyruvate-driven production of \(\alpha\)-ketoglutarate. Consequently, we measured the abundance of \(\alpha\)-ketoglutarate and hydroxylated collagen upon ALT2 inhibition and found that both were decreased (Extended Data Fig. 5c–f). Thus, we concluded that pyruvate drives the production of \(\alpha\)-ketoglutarate through ALT conversion.

Next, we investigated whether \(\alpha\)-ketoglutarate is a carbon donor for generating the P4HA substrate proline, or whether \(\alpha\)-ketoglutarate metabolically activates P4HA through enzyme kinetics (Extended Data Fig. 6a). We excluded the first mechanism by inhibiting the conversion of \(\alpha\)-ketoglutarate to proline by silencing pyrroline-5-carboxylate synthase (P5CS, which is also known as ALDH18A1) (Extended Data Fig. 6b, c). We then investigated whether \(\alpha\)-ketoglutarate could be a metabolic activator of P4HA (Extended Data Fig. 6a). Metabolic activation depends on metabolite substrate- and product-driven enzyme activity\(^{10}\)—that is, high substrate metabolite concentrations activate flux through a metabolic enzyme, whereas high product metabolite concentrations have an inhibitory effect. Thus, if \(\alpha\)-ketoglutarate is a metabolic activator of P4HA activity, succinate—as a coproduct of the reaction—should impair P4HA activity. Therefore, we added succinate to breast cancer spheroids and measured hydroxylated collagen in the presence of pyruvate. In accordance with the metabolic activator mechanism, we found that succinate significantly decreased the abundance of hydroxylated collagen, whereas \(\alpha\)-ketoglutarate on top of succinate rescued it (Fig. 2c). These findings support the notion that P4HA is metabolically regulated by \(\alpha\)-ketoglutarate. Notably, non-transformed cells were not significantly or only mildly affected (Extended Data Fig. 9a, b), the metastatic burden was substantially reduced upon pyruvate uptake and metabolism inhibition (Fig. 4h, i and Extended Data Fig. 9c–e). In accordance, treatment with \(\alpha\)-ketoglutarate upon MCT2 inhibition fully restored the higher metastatic burden (Fig. 4 and Extended Data Fig. 9f). Therefore, we conclude that inhibition of pyruvate metabolism is sufficient to impair metastatic growth in the lung.

Taken together, these results demonstrate that pyruvate serves as a pivotal nutrient in the lung metastatic niche to promote cancer-cell-dependent modification of the ECM that supports metastatic growth (Fig. 4k). Moreover, we provide evidence that nutrients that fuel metabolic regulation can be decoupled from nutrients that fuel carbon biosynthetic needs. This discovery enables targeting regulation by metabolite concentrations, which was so far considered to be undruggable\(^{12}\). In conclusion, our data suggest that targeting pyruvate uptake can normalize aberrant collagen remodelling in the lung metastatic niche and thus impair metastatic growth.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-0977-x.

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Author contributions I.E. performed most experiments and analysed all data. Key in vitro experiments were reproduced by M.v.G., M.R. and C.E.-N. M.R. helped with microscopy analysis, fibroblast and western blot analysis. D.B. and G.D. helped with western blot analysis and/or in vivo experiments. M.v.G. helped with the imaging of the tissue sections and helped with in vitro sample collection. S.S. and S.T. performed hydroxyproline measurements and collagen synthesis measurements. R.B. and C.V. helped with CRISPR and overexpression construct designs. C.E.-N. performed the experiment with the HCC70 cell line. E.V. helped with the interpretation of in vivo ECM remodelling data. G.C. provided advice on ECM remodelling. I.E. and S.-M.F. designed the study and wrote the manuscript. S.-M.F. conceived and supervised the study and obtained funding.

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METHODS

Cell culture. MCF10A cells expressing H-RASV12 (MCF10A H-RASV12) as well as control cells expressing an empty pLA vector (MCF10A) were generated as previously described11. MCF10A and MCF10A H-RASV12 cells were grown in Dulbecco’s modified Eagle’s medium/F12 (DMEM/F12) supplemented with 5% horse serum, 1% penicillin (50 units per ml), 1% streptomycin (50 μg ml−1), 0.5 μg ml−1 hydrocortisone, 100 ng ml−1 cholera toxin, 10 μg ml−1 insulin and 20 ng ml−1 recombinant human EGF. MCF10A and MCF10A H-RASV12 cells were cultured in 0.5 mM pyruvate. Because MCF10A H-RASV12 cells undergo growth inhibition upon pyruvate depletion, we used 0.02 mM pyruvate for these cells instead of complete pyruvate depletion. MCF7 cells, HCC70 cells, primary human skin-derived fibroblasts, immortalized human mammary myofibroblasts and immortalized human mammary cancer-associated myofibroblasts14 were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin (50 units per ml), 1% streptomycin (50 μg ml−1) and with or without 1 mM pyruvate. 4T1 and EMT6.5 cells were cultured in Roswell Park Memorial Institute medium (RPMI) with 10% fetal bovine serum, 1% penicillin (50 units per ml), 1% streptomycin (50 μg ml−1) and with or without 1 mM pyruvate. All cell lines were cultured in spheroids as described previously15. Growth-factor-reduced Matrigel (3%) without phenol red was added into cold DMEM/F12 medium before seeding by using tips precooled to −20 °C. Three-dimensional cultures were performed as described previously15,16. All cell lines were confirmed to be mycoplasma-free by Mycoalert detection kit (Lonza). MCF10A, MCF7, HCC70 and 4T1 cell lines were purchased from ATCC. MCF10A H-RASV12, MCF7 and HCC70 cells, respectively. Malate and citrate were purchased from Sigma-Aldrich and used at a concentration of 50 μM, TGFα and TGFβ were purchased from Peprotech and used at a concentration of 1.5 mM. Effective transport of supplemented metabolites into cells, respectively. Malate and citrate were purchased from Sigma-Aldrich and used at a concentration of 50 μM. In Figs. 2c, 3b, 5b, 9a, and 10a, MCF10A cells expressing H-RASV12 (MCF10A H-RASV12) as well as control cells expressing an empty pLA vector (MCF10A) were generated as previously described13. MCF10A H-RASV12 cells were selected with puromycin (1 μg ml−1). As a control, a CRISPR line lacking the gRNA for human MCT2 or mouse Mct2 was generated. CRISPR-based knockdown was confirmed by western blot (Extended Data Fig. 10).

Overexpression. The P4HA1-expressing line was generated by cloning the 1,605-bp coding domain sequence of P4HA1 into the lentiviral pLVX-ires vector (Clontech, 623185) through Gibson cloning. The sequence of P4HA1 was bought as a gBlock from IDT. Lentiviral particles were produced in HEK293 cells. MCF10A H-RASV12 cells were selected with hygromycin (600 μg ml−1). As a control, an empty lentiviral pLVX-IRES vector was generated. Overexpression was confirmed by qPCR (Extended Data Fig. 7b) and western blot (Extended Data Fig. 10).

Western blot analysis. Cells were collected and then lysed in RIPA lysis and extraction buffer (Thermo Scientific) supplemented with proteinase (Roche, 11836153001) and phosphatase (PhosSTOP, Sigma, 4906884501). Protein amounts were measured using a Pierce BCA protein assay kit (Thermo Scientific). Subsequently, 25–40 μg of protein was loaded on a NuPAGE 4−12% denaturing Bis-Tris gel and transferred to a nitrocellulose membrane (Thermo Scientific). Membranes were incubated overnight at 4 °C with either MCT2 (LabNed, 013512; 1:200 dilution), GPT2 (Santa Cruz, 398383; 1:500 dilution), P5CS (Santa Cruz, 51543; 1:500 dilution), DHG (Abcam, 153973; 1:000 dilution), P4HA1 (Abcam, 59497; 1:0000 dilution), γ-actin (Sigma, A5441; 1:10,000 dilution) or ERK1/2 (Cell Signalling Technology, 1:5,000 dilution) primary antibodies. The day after the membranes were incubated with mouse (Cell Signalling Technology, 7067; 1:5,000 dilution), rabbit (Cell Signalling Technology, 7067; 1:5,000 dilution) or goat (Abcam, 6566; 1:5,000 dilution) secondary antibodies, and bound antibodies were visualized using Pierce ECL reagent (ThermoFisher Scientific).

RNA isolation and qPCR. Total RNA was isolated with the PureLink RNA Mini kit (Life Technologies). RNA was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies). The relative levels of transcripts compared to the control RPL19 were determined by qPCR using SYBR Green PCR Master Mix (Life Technologies) and specific primers on a 7500 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.RPL19: forward, 5′-ATGGTCTATGGTGCTTAC-3′; reverse, 5′-AGATGCTAGCTCCGAGAA-3′. P4HA1: forward, 5′-GGCGTCTGTTTAGATCTGTTC-3′; reverse, 5′-GGCGATCCGCTCTGTTAGT-3′. DHG: forward, 5′-TTTCGATGCGAGAGGAGGAC-3′; reverse, 5′-AGGTGCCGACTGCTGAT-3′. P5CS: forward, 5′-CTTACGAGAGGAGGACG-3′; reverse, 5′-ATGGGAGGACTGCTGAT-3′. P4HA2: forward, 5′-GGAGAAGGAGGTGGCTGCT-3′; reverse, 5′-GGAGAAGGAGGTGGCTGCT-3′. ALDH18A1: forward, 5′-CCACCCACTTA-3′; reverse, 5′-GGCTGAAAACATCC-3′. ALDH18A1 (also known as ALDH1A1): forward, 5′-AGCTCCCCCTGCCAGTTAGT-3′; reverse, 5′-AAAGTCTGCAACCTTTTT-3′. ALDH18A1: forward, 5′-GATGCTGCTGCTGCTGCTGCTGCT-3′; reverse, 5′-GGAGAAGGAGGTGGCTGCT-3′. P4HA2: forward, 5′-GGAGAAGGAGGTGGCTGCT-3′; reverse, 5′-GGAGAAGGAGGTGGCTGCT-3′. P5CS: forward, 5′-CCACCCACTTA-3′; reverse, 5′-GGCTGAAAACATCC-3′. ALDH18A1: forward, 5′-GATGCTGCTGCTGCTGCTGCTGCT-3′; reverse, 5′-AAAGTCTGCAACCTTTTT-3′. ALDH18A1: forward, 5′-GATGCTGCTGCTGCTGCTGCTGCT-3′; reverse, 5′-AAAGTCTGCAACCTTTTT-3′. ALDH18A1: forward, 5′-GATGCTGCTGCTGCTGCTGCTGCT-3′; reverse, 5′-AAAGTCTGCAACCTTTTT-3′. ALDH18A1: forward, 5′-GATGCTGCTGCTGCTGCTGCTGCT-3′; reverse, 5′-AAAGTCTGCAACCTTTTT-3′.

Mass-spectrometry analysis. The quenching and extraction procedure of three-dimensional spheroids was performed as described previously14,15. Pseudo-labeling steady state after 5 days of labelling was verified experimentally (Extended Data Fig. 10f, g). Metabolite abundances and 13C labelling patterns were analysed by either gas or liquid chromatography–mass spectrometry. Metabolites for liquid chromatography–mass spectrometry analysis were extracted, derivatized and measured as described previously14,15. Metabolites for liquid chromatography–mass spectrometry analysis were derivatized and measured as described previously14,15. In brief, metabolites were reconstituted in 60% acetonitrile. Metabolites were measured using a 1290 Infinity II HPLC (Agilent) coupled to a 6470 triple quadrupole mass spectrometer (Agilent). Samples were injected onto an IHD-IC-Fusion(P) column with the above-mentioned solvents. The solvent, composed of acetonitrile and ammonium acetate (pH 9.3, 10 μM), was used at a flow rate of 0.100 ml min−1. Data analysis was performed with Msd chromatography Data Analysis (v.2.02.1.431) or Agilent MassHunter (v.B.0802 Build 8.2.8260.0) followed by an in-house-developed MATLAB script.

Hydroxylated collagen and collagen stability. We assessed hydroxylated collagen by measuring the total hydroxyproline content. This assumes that the majority of hydroxyproline content is from collagen I. Hydroxyproline content was quantified by a colorimetric protocol as described previously19. Spheroids or extracts from tissue were washed once in PBS and hydrolysed for 3.5 h at 135 °C in 6 N HCl. Thereafter, samples were vacuum-evaporated and dissolved at 135 °C in 6 N HCl. Thereafter, samples were vacuum-evaporated and dissolved.
in deamineralized water. Hydroxyproline residues were oxidized by adding chloramine-T (Sigma-Aldrich), followed by the addition of Ehrlich's aldehyde reagent (mixture of p-dimethylaminobenzaldehyde, n-propanol and perchloric acid) and incubation of the samples at 65°C for chromophore development. A standard curve was made to calculate the absolute amount of hydroxyproline per sample, which was normalized to the protein content of a parallel sample (spheroids) or the same sample (tissue) determined by BCA protein assay.

To assess collagen stability via resistance to MMP-mediated degradation, breast cancer spheroids were incubated with MMP9 (0.4 ng ml⁻¹; Bio-Techne) for 24 h at 37°C before hydrolysis of cells and supernatant. Subsequently, the hydroxyproline content of the cells and the supernatant was measured as described above.

**Total synthesis of collagen and protein.** Total collagen and protein synthesis was quantified by incubating cells with 20 μCi ml⁻¹ 1-2,3,4,5-⁴H-hydroxyproline (PerkinElmer), as described previously. In brief, after overnight labelling, cells were lysed in extraction buffer (11% acetic acid in H₂O with 0.25% BSA) and collagens were precipitated by the addition of 20% trichloroacetic acid. Radioactivity was determined by liquid scintillation counting, and normalized for protein content.

**Protein synthesis.** Protein synthesis was analysed using the Click-IT HPG Alexa Fluor 488 Protein Synthesis Assay Kit (ThermoFisher Scientific) according to the manufacturer's instructions. In brief, tumour spheroids were incubated with 50 μM Click-IT HPG overnight in culture medium. Breast cancer spheroids were then fixed with 3.7% formaldehyde for 15 min at room temperature, washed and permeabilized with 0.5% Triton X-100 for 20 min at room temperature. Subsequently, cells were incubated with Click-IT reaction cocktail for 30 min at room temperature protected from light, washed and fluorescence was detected by flow cytometry. This assay assumes that the majority of methionine is incorporated into non-collagen protein.

**In vivo pharmacological and genetic inhibition and metabolism.** All animal experiments were approved by the local authorities in compliance with all relevant ethical regulations (including but not limited to tumour size). For injection models, mice were randomized before injection of cancer cell lines. All samples were analysed blinded. Sample size was determined using power calculations with B = 0.8 and P = 0.05 based on preliminary data and in compliance with the 3R system: Replacement, Reduction, Refinement.

Six-week-old female BALB/c mice were inoculated with 4T1 or EMT6.5 cells either in the mammary fat pad (m.f.; 1 × 10⁶ cells) or intravenously (i.v.; 1 × 10⁶ cells). α-Cyano-4-hydroxyniminonic acid treatment was started after 4 days when the primary tumour nodules were established and palpable. α-Cyano-4-hydroxyniminonic acid (60 mg per kg) was intraperitoneally injected daily into cancer-bearing mice. α-Cyano-4-hydroxyniminonic acid was dissolved in a mix containing 1.5% DMSO, 60% 5-cycloextrin, 35% polyethylene glycol (PEG) and 5% ethanol and pH neutralized with NaOH. The same mix without drug was injected as a vehicle to control animals. α-Ketoglutarate was intraperitoneally injected daily at a concentration of 50 mg per kg. Treated and control mice were randomly chosen. Mice were euthanized after 16 days of treatment. 4T1 Mtx2 grNA m.f. and EMT6.5 Mtx2 grNA m.f. models were euthanized after 28 days. All i.v. injected models were euthanized after 10 days. Mtx2 grNA 2 and Alf2 shRNA 1 were used for in vivo experiments. The animal study complies with ethical regulations and was approved by the KU Leuven ethics committee.

Metabolites were collected, extracted and analysed as described previously. Metabolites in the blood plasma were measured in the 4T1 m.f. model after 16 days of treatment with α-cyano-4-hydroxyniminonic acid (60 mg per kg; started 5 days after primary tumour initiation) or vehicle (Fig. 4a (left and middle)). α-Ketoglutarate in the metastasis tissues was measured in the 4T1 m.f. model after 2 days of treatment with α-cyano-4-hydroxyniminonic acid (60 mg per kg; started 18 days after primary tumour initiation; Fig. 4a (right)). Hydroxyproline was measured in the metastasis tissues after 16 days of treatment with α-cyano-4-hydroxyniminonic acid (60 mg per kg; started 5 days after primary tumour initiation) and/or α-ketoglutarate (50 mg per kg; started 5 days after primary tumour initiation) or vehicle (Fig. 4b (left), d). Moreover, hydroxyproline was measured 28 days (Fig. 4b (right, i.v. model)) or 10 days (Fig. 4b (right, i.v. model)) after injection of genetically modified cells. Mice were euthanized using nembutal, the breast tumours and lung metastases were placed in cold saline, dissected in less than 3 min and immediately frozen using a liquid-nitrogen-cooled Biosqueezer (Biospec Products). The tissue was weighed (10–15 mg) and pulverized (Cryomill, Retsch) under liquid-nitrogen conditions. The pulverized tissue was used to measure α-ketoglutarate and hydroxyproline as described above. In addition, 20 μl of blood plasma was extracted and analysed as described above. Humane end points were determined as follows: tumour size of 2 cm³. Following additional symptoms were monitored and upon detection of one of the symptoms, the animal was euthanized: loss of ability to ambulate, laboured respiration, surgical infection or weight loss over 10% of initial body weight. Housing and experimental animal procedures were approved by the Institutional Animal Care and Research Advisory Committee of KU Leuven, Belgium.

**Picro-Sirius red, and haematoxylin and eosin staining.** Treatments were performed as described above, that is, pharmacological MCT2 inhibition was initiated upon palpable primary tumour, whereas genetic Mtx2 inhibition was present already during tumour initiation. Tissues were collected at the same time points as described above. For Sirius red and haematoxylin and eosin staining of pulmonary metastases, dissected lung samples were gently infused via the trachea with 10% formalin and then processed as previously described. Then, 5-μm thick sections obtained from the resulting paraffin blocks were stained with either picro-Sirius red or haematoxylin and eosin. Because picro-Sirius red stains thinner fibres in green and thicker fibres in red, and collagen III fibres are thinner than collagen I fibres, the assumption is that green is mainly collagen III whereas red is mainly collagen I. Picro-Sirius red slides were analysed with polarized microscope Image M2 Carl Zeiss at 20 × magnification. A sufficient number of images was acquired for each metastasis to cover the whole metastatic area, after which the GIMP Image Manipulation Program (GIMP 2.10.8) was used to manually stitch all images belonging to the same metastasis together. The composite images were analysed with ImageJ. In brief, the polarized light images were split by RGB (red, green, blue), then the signals in the green and red channels were quantified. Only metastases with similar size were analysed. One to four similarly sized metastases per mouse were analysed. Native collagen and highly vascularized metastases were excluded from analysis.

Metastatic area and the number of metastases were quantified by ZEN blue software (2011). Metastatic index was calculated by dividing the metastatic area by the primary tumour weight. Only mice with a primary tumour of 0.8 g or greater were analysed. All i.v.-injected animals were analysed. The animal study complied with ethical regulation and was approved by the KU Leuven ethics committee.

**Statistical analysis and software.** Statistical data analysis was performed using GraphPad Prism version 7.0 (GraphPad Software) on n ≥ 3 biological replicates. Details on statistical tests and post-tests are presented in the figure legends. In brief, two-tailed unpaired Student's t-tests were performed on n ≥ 3 biological replicates. Two-way ANOVA was performed in Fig. 3a as described in the correspondent figure legend. Sample size for all experiments was chosen empirically. Independent experiments were pooled and analysed together whenever possible as detailed in figure legends. Detection of outliers was performed using Grubb's test in GraphPad. All graphs show mean ± s.d. or s.e.m. as indicated in the figure legends.

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

**Data availability**

The authors declare that all data supporting the findings of this study are available within the paper, Extended Data Figs. 1–10, the Source Data (of Figs. 1–4 and Extended Data Figs. 1–7, 9, 10) or from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | Pyruvate is required for three-dimensional but not two-dimensional growth of breast cancer cells. a, b, Growth curves (two-dimensional, left) and representative pictures (three-dimensional, right) of human MCF10A H-RAS^V12 and mouse 4T1 cells cultured in medium with or without pyruvate, glucose or glutamine. c, Representative pictures of MCF10A H-RAS^V12 and MCF10A spheroids in the presence or absence of pyruvate or 0.5% supplemented ECM (Matrigel). Analysis was performed at day 5. The number of biological replicates for each experiment was n = 3. Data are mean ± s.d. of biological independent samples. Scale bars, 150 μm. d, Cellular pyruvate, α-ketoglutarate and hydroxyproline metabolism. Enzymes are depicted in bold and italics. ALT2, mitochondrial alanine aminotransferase; GDH, glutamate dehydrogenase; MCT2, monocarboxylate transporter 2; MPC, mitochondrial pyruvate carrier; P4HA, collagen prolyl-4-hydroxylase; OH-proline, hydroxyproline. Only selected reactions are depicted.
Extended Data Fig. 2 | Pyruvate depletion does not affect collagen synthesis. a, Total collagen and protein synthesis (left) as well as protein synthesis (right) in human and mouse breast cancer spheroids with and without pyruvate. CPM, counts per min. Total collagen and protein synthesis was assessed by incorporation of radioactive proline into collagen and protein, whereas protein synthesis was assessed by fluorescently labelled methionine incorporation into protein. The latter is more specific for protein synthesis since methionine is only to a minor extent incorporated into collagen. Changes that occur in both parameters indicate alterations in protein synthesis, whereas changes that occur only in total collagen and protein synthesis indicate alterations in collagen synthesis. Our data indicate that collagen synthesis was not altered by pyruvate depletion, because either total collagen and protein synthesis were not altered or both parameters were altered to a similar extent in the tested cell lines. n = 3. b, Relative abundance of collagen I and III and representative three-dimensional reconstruction in human MCF10A H-RasV12 and mouse 4T1 breast cancer spheroids with and without pyruvate measured by immunofluorescence. n = 5. Collagen I and III are major collagen species in breast cancer. Blue, DAPI-stained nuclei; red, collagen I; green, collagen III. The total fluorescence intensity was measured in each microscopy field and normalized over the cell number, scored as the number of DAPI-stained nuclei. Five microscopy fields were averaged for each sample. Relative fluorescence intensities per cell are depicted, normalized to the control condition. The solid line indicates the median, the box extends to the 25th and 75th percentiles, the whiskers span the smallest and the largest values. Data are mean ± s.d. of biological independent samples unless otherwise noted. Two-tailed unpaired Student’s t-test.
Extended Data Fig. 3 | Pyruvate drives collagen hydroxylation.

a, Pyruvate, lactate and glucose uptake/secretion in human MCF10A H-RASV12 breast cancer spheroids treated with MCT2 inhibitor (α-cyano-hydroxycinnamic acid; 1.5 mM). These data show that the used inhibitor impairs pyruvate uptake, but not lactate secretion or glucose uptake.

b, c, Hydroxylated collagen was assessed by measuring hydroxyproline in human (MCF10A H-RASV12, MCF7 and HCC70) and mouse (4T1) breast cancer spheroids treated with a MCT2 inhibitor (α-cyano-hydroxycinnamic acid; 1.5 mM), or treated with the MPC inhibitor UK5099 (50 μM) in the presence of pyruvate. The number of biological replicates for each experiment was n = 3. Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student’s t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Pyruvate drives collagen hydroxylation via \(\alpha\)-ketoglutarate. a, Heat map representing metabolite changes in MCF10A H-RAS\(^{V12}\) spheroids in the presence or absence of pyruvate measured by mass spectrometry. Blue, significantly reduced metabolites upon pyruvate depletion. \(n = 3\). b, Intracellular abundance of pyruvate, \(\alpha\)-ketoglutarate (\(\alpha\)-KG), citrate and malate in human MCF10A H-RAS\(^{V12}\) breast cancer spheroids with and without pyruvate. \(n = 3\). c, Hydroxylated collagen was assessed by measuring hydroxyproline in MCF10A H-RAS\(^{V12}\) spheroids in the presence or absence of pyruvate upon addition of lactate (2 mM), alanine (2 mM), glutamate (2 mM) or cell-permeable \(\alpha\)-ketoglutarate (1.5 mM). \(n = 3\). d, Intracellular abundance of \(\alpha\)-ketoglutarate, citrate and malate in human MCF10A H-RAS\(^{V12}\) breast cancer spheroids upon supplementation of cell-permeable \(\alpha\)-ketoglutarate (1.5 mM), citrate (5 mM) or malate (5 mM). \(n = 3\). e, Relative change in hydroxylated collagen was assessed by measuring hydroxyproline in human (MCF10A, MCF7 and HCC70) and mouse (4T1 and EMT6.5) breast cancer spheroids in the absence of pyruvate with or without cell-permeable \(\alpha\)-ketoglutarate (1.5 mM). Data are normalized to controls. Dashed line indicates the level of hydroxylated collagen in control conditions with pyruvate. \(n = 3\) for MCF10A and EMT6.5; \(n = 6\) for MCF7 and HCC70; \(n = 9\) for MCF10A H-RAS\(^{V12}\) and 4T1. f, Relative change in hydroxylated collagen was assessed by measuring hydroxyproline in MCF10A H-RAS\(^{V12}\) spheroids treated with the MCT2 inhibitor \(\alpha\)-cyano-4-hydroxycinnamic acid (1.5 mM) upon addition of cell-permeable \(\alpha\)-ketoglutarate (1.5 mM) in the presence of pyruvate. Data are normalized to control. Dashed line indicates the level of hydroxylated collagen in the control condition. \(n = 3\). Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student’s \(t\)-test.
Extended Data Fig. 5 | Pyruvate to alanine conversion drives α-ketoglutarate production. a, Carbon contribution of $^{13}$C$_5$-glutamine, $^{13}$C$_6$-glucose and $^{13}$C$_3$-pyruvate to alanine and α-ketoglutarate assessed by $^{13}$C tracer analysis. n = 3. b, Alanine uptake/secretion in MCF10A H-RAS$^{V12}$ spheroids with and without pyruvate was measured by mass spectrometry analysis of the medium. n = 3. c–f, Intracellular abundance of α-ketoglutarate and hydroxylated collagen in human and mouse breast cancer spheroids upon treatment with the transaminase inhibitor aminooxyacetate (AOA) (0.8 mM), the glutamate dehydrogenase (GDH) inhibitor epigallocatechin gallate (EGCG) (50 μM), transduced with a lentiviral vector with shRNA against either mitochondrial ALT2 or Alt2 (KD), GDH (KD) or scrambled control sequence in the presence of pyruvate. n = 3 for EGCG and AOA treatment (c, d); n = 9 for control shRNA, n = 6 for GDH shRNA 1 and 2 and n = 3 for ALT2 shRNA 1 and 2 (MCF10A H-RAS$^{V12}$, e, f); n = 3 for control shRNA and Alt2 shRNA 1 and 2 (4T1; e, f). If ALT activity majorly contributes to α-ketoglutarate generation, EGCG (which inhibits the pyruvate-independent conversion of glutamate to α-ketoglutarate via GDH), should have a minor effect on α-ketoglutarate abundance and hydroxylated collagen. Indeed, we found that this was the case. Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student’s t-test.
Extended Data Fig. 6 | α-Ketoglutarate metabolically regulates P4HA activity in cancer cells. a, Schematic representation of the metabolic regulation and carbon donor mechanisms by which α-ketoglutarate can regulate collagen hydroxylation. Solid lines indicate metabolite conversion; dashed lines indicate metabolic regulation. Enzymes are depicted in bold and italics. P4HA, collagen prolyl-4-hydroxylase; P5CS, pyrroline-5-carboxylate synthase. b, c, Relative change in intracellular abundance of proline and hydroxylated collagen in MCF10A H-RAS V12 spheroids transduced with a lentiviral vector with shRNA against either P5CS (KD) or a scrambled control sequence with or without cell-permeable α-ketoglutarate (1.5 mM) in the presence or absence of pyruvate normalized to the control condition. If the carbon donor mechanism occurs, it is expected that the abundance of proline decreases in P5CS knockdown spheroids and that they no longer respond to rescue by α-ketoglutarate upon pyruvate depletion. However, we observed that proline abundance did not significantly change in P5CS knockdown spheroids. Moreover, α-ketoglutarate addition still significantly increased hydroxylated collagen to a similar extent as pyruvate. n = 3 replicates (b); n = 6 control shRNA; n = 3 P5CS shRNA 1 and 2. d, Hydroxylated collagen was assessed by measuring hydroxyproline in human (myo) fibroblasts in the presence or absence of pyruvate with or without cell-permeable α-ketoglutarate (1.5 mM) and/or cell-permeable succinate (1.5 mM). n = 3 human primary skin-derived fibroblasts; n = 4 human immortalized mammary and cancer associated myofibroblasts. e, Hydroxylated collagen was assessed by measuring hydroxyproline in human (myo) fibroblasts treated with the MCT2 inhibitor α-cyano-4-hydroxycinnamic acid (1.5 mM), the MPC inhibitor UK5099 (50 μM) or the transaminase inhibitor AOA (0.8 mM) in the presence of pyruvate. n = 3. f, Intracellular abundance of α-ketoglutarate in the presence or absence of pyruvate in human fibroblasts. n = 3. Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student's t-test.
Extended Data Fig. 7 | Metabolic regulation of P4HA activity is independent of its known transcriptional regulation. a, Absolute levels of P4HA1, P4HA2 and P4HA3 in human MCF10A H-RasV12 breast cancer spheroids in the presence of pyruvate. b, Relative change in P4HA1 gene expression upon pyruvate depletion as well as P4HA1 overexpression (OE) in normoxia, P4HA1 expression in hypoxia (1% oxygen), upon TGFβ addition and 50 μM IOX2 treatment normalized to the control condition with pyruvate. c, Relative P4HA1 or P4ha1 gene expression in human (MCF7 and HCC70) and mouse (4T1) breast cancer spheroids in the presence (normoxia) or absence (hypoxia (1% oxygen); IOX2 (50 μM); TGFβ (12 ng ml⁻¹)) of pyruvate normalized to the normoxia condition with pyruvate. d, Hydroxylated collagen was assessed by measuring hydroxyproline in human (MCF10A H-RasV12, MCF7 and HCC70) and mouse (4T1) breast cancer spheroids treated with TGFβ (12 ng ml⁻¹) or IOX2 (50 μM) with or without pyruvate or upon addition of cell-permeable α-ketoglutarate (1.5 mM). The number of biological replicates for each experiment was n = 3. Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student’s t-test.
Extended Data Fig. 8 | Functional collagen deposition decreases in the lung metastatic niche upon pyruvate metabolism inhibition. Representative pictures of functional collagen of lung metastases tissue based on picro-Sirius red staining and polarized light microscopy. Red predominantly indicates thick collagen I fibres and green predominately indicates thin collagen III fibres. 

a, 4T1 model (m.f.) upon pharmacological inhibition of MCT2 (α-cyano-4-hydroxycinnamic acid; 60 mg per kg, i.p.).
b, 4T1 (m.f.) and EMT6.5 (i.v.) models upon genetic inhibition of Mct2.
c, 4T1 model (m.f.) upon pharmacological inhibition of MCT2 (α-cyano-4-hydroxycinnamic acid; 60 mg per kg, i.p.) with or without treatment with cell-permeable α-ketoglutarate (50 mg per kg, i.p.).
d, 4T1 model (i.v.) upon genetic inhibition of Alt2. Scale bars, 50 μm.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Metastatic burden decreases independently of primary tumour volume upon pyruvate metabolism inhibition.

a, Primary tumour volume over time and final tumour weight upon pharmacological inhibition of MCT2 (α-cyano-4-hydroxycinnamic acid; 60 mg per kg, i.p.) with or without treatment with cell-permeable α-ketoglutarate (50 mg per kg, i.p.) in the 4T1 model (m.f.). n = 23 (vehicle, 3 cohorts), n = 24 (MCT2 inhibitor, 2 cohorts), n = 10 (MCT2 inhibitor and α-ketoglutarate). b, Primary tumour volume over time and final tumour weight upon genetic inhibition of MCT2 in the 4T1 model (m.f.). n = 11 (4T1 control), n = 7 (4T1 Mct2 gRNA). c, Representative pictures of tissue from lung metastases upon genetic inhibition of Mct2 in the 4T1 model (m.f.) based on haematoxylin and eosin staining. d, Representative pictures of tissue from lung metastases upon genetic inhibition of Mct2 in the EMT6.5 model (i.v.) based on haematoxylin and eosin staining. e, Representative pictures of tissue from lung metastases upon genetic inhibition of Alt2 in the 4T1 model (i.v.) based on haematoxylin and eosin staining. f, Representative pictures of tissue from lung metastases upon pharmacological inhibition of MCT2 (α-cyano-4-hydroxycinnamic acid; 60 mg per kg, i.p.) with or without treatment with cell-permeable α-ketoglutarate (50 mg per kg, i.p.) in the 4T1 model (m.f.) based on haematoxylin and eosin staining. The much milder effect of MCT2 inhibition compared to the previously described P4HA inhibition on primary tumour growth could be explained by our previous observation that pyruvate is less available to primary breast cancers than to lung metastases. Arrow heads indicate metastasis tissue. Data are mean ± s.e.m. from different mice. Two-tailed unpaired Student's t-test. Scale bars, 0.5 cm.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Protein and RNA expression of genetically modified breast cancer cells. a, Western blot analysis of MCT2 in human (MCF10A H-RASV12 and MCF7) and mouse (4T1 and EMT6.5) breast cancer cells infected with either a control gRNA or two different MCT2 or Mct2 gRNAs normalized to the control condition. Human positive/negative control: H460/MDA-MB-468; mouse positive/negative control: testis/lung. b, Western blot analysis and relative gene expression of GDH in human MCF10A H-RASV12 breast cancer cells infected with either a control shRNA or two different GDH shRNAs normalized to the control condition. c, Western blot analysis and relative gene expression of ALT2 in human (MCF10A H-RASV12) and mouse (4T1) breast cancer cells infected with either a control shRNA or two different ALT2 or Alt2 shRNAs normalized to the control condition. d, Western blot analysis and relative gene expression of P5CS in human MCF10A H-RASV12 breast cancer cells infected with either a control shRNA or two different P5CS shRNAs. e, Western blot analysis of P4HA in human MCF10A H-RASV12 breast cancer cells infected with either a control or an P4HA-overexpression vector. f, g, Time-resolved contribution of 13C6-glucose, 13C5-glutamine and 13C3-pyruvate to α-ketoglutarate and alanine in human MCF10A H-RASV12 breast cancer spheroids. The number of biological replicates for each experiment was n = 3. Data are mean ± s.d. of biological independent samples. Two-tailed unpaired Student’s t-test. For gel source data, see Supplementary Fig. 1.
Reporting Summary

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

- [ ] Confirmed
- [X] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [ ] The statistical test(s) used AND whether they are one- or two-sided
- [ ] Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- [ ] A description of all covariates tested
- [ ] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [ ] A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- [ ] For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever possible.
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [ ] Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- [ ] Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | Mass spectrometry data were collected using MSDF Chemstation Data Analysis (vE.02.0.2.1431) or Agilent Mass Hunter (vB.0802 Build 8.2.8260.0) followed by an in house developed Matlab script. |
|-----------------|-------------------------------------------------------------------------------------------------------------------|
| Data analysis   | Mass spectrometry data were analyzed using MSD Chemstation Data Analysis (vE.02.0.2.1431) or Agilent Mass Hunter (vB.0802 Build 8.2.8260.0) followed by an in house developed Matlab script. Metastatic area and metastases number were quantified by Zen Blue software (2011). Collagen intensity was quantified with Imaris Image Analysis Software 8 (Bitplane). GNU Imagin Manipulation Program (GIMP 2.10.8) was used to manually stitch all picro-sirius images belonging to the same metastasis together. The composite images were analyzed with Image J 1.45. Microsoft excel 2013 was used for data output. Statistical data analysis was performed using GraphPad Prism 7 software. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

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

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

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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

Life sciences study design

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

Sample size

In vitro sample sizes were based on previous similar studies that have given statistical results. For in vivo experiments, sample size was determined using power calculations with $\alpha=0.8$ and $\beta=0.05$, based on preliminary data and respects the limited use of animals in line with the 3R system: replacement, reduction, refinement.

Data exclusions

Detection of outliers was performed using Grubbs's test in Graphpad.

Replication

All experiments were performed at least in triplicates. All attempts at replications were successful.

Randomization

Mice were randomized into control and treatment groups.

Blinding

Mice were given a number prior to data collection and analysis. Data was collected and analyzed, and subsequently grouped in the corresponding cohorts for statistical analysis.

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| [x] Involved in the study       | [x] Involved in the study |
| [x] Unique biological materials | [ ] ChIP-seq |
| [x] Antibodies                  | [x] Flow cytometry |
| [x] Eukaryotic cell lines       | [x] MRI-based neuroimaging |
| [x] Palaeontology               |         |
| [x] Animals and other organisms |         |
| [x] Human research participants |         |

Antibodies

Antibodies used

Collagen staining was performed with either anti-Collagen I (Abcam, Ab34710) or anti-Collagen III (Abcam, Ab7778). Alexa Fluor 555 (Life technologies, A31272) was used as conjugated secondary antibody. Western blot analysis was performed with the following antibodies: MCT2 (Labmed 0315312), GPT2 (Santa Cruz, 393838), PSCS (Santa Cruz, 515443), GDH (Abcam, 153973), P4HA1 (Abcam, 59497), B-Actin (Sigma, A5441) and ERK1/2 (Cell signaling, 46955).

Validation

Antibody were used as recommended in the respective data-sheets. MCT2 antibody was validated based on positive and negative control cell lines.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) MCF10A, MCF7, HCC70 and 4T1 cell lines were purchased from ATCC. The EMT6.5 cell line was kindly provided by professor Robin Anderson (Peter MacCallum cancer center). Fibroblasts were kindly provided by Prof. Ludo Van Den Bosch (ViB). Myofibroblasts were kindly provided by Prof. Akira Orimo (Juntendo University).

Authentication MCF10A, MCF7 and HCC70 cell lines were validated by DNA fingerprinting.

Mycoplasma contamination All cell lines were confirmed to be mycoplasma free by Mycoalert detection kit (Lonza).

Commonly misidentified lines (See ICLAC register) No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals Six weeks old female BALB/c mice were injected with mouse breast cancer cells through either mammary fat pad injection or intravenously. Mice were sacrificed after 2-4 weeks.

Wild animals No wild animals were used.

Field-collected samples No field-collected samples were used.