Increased lipogenesis has been linked to an increased cancer risk and poor prognosis; however, the underlying mechanisms remain obscure. Here we show that phosphatidic acid phosphatase (PAP) lipin-1, which generates diglyceride precursors necessary for the synthesis of glycerolipids, interacts with and is a direct substrate of the Src proto-oncogenic tyrosine kinase. Obesity-associated microenvironmental factors and other Src-activating growth factors, including the epidermal growth factor, activate Src and promote Src-mediated lipin-1 phosphorylation on Tyr398, Tyr413 and Tyr795 residues. The tyrosine phosphorylation of lipin-1 markedly increases its PAP activity, accelerating the synthesis of glycerophospholipids and triglyceride. Alteration of the three tyrosine residues to phenylalanine (3YF-lipin-1) disables lipin-1 from mediating Src-enhanced glycerolipid synthesis, cell proliferation and xenograft growth. Re-expression of 3YF-lipin-1 in PyVT;Lpin1−/− mice fails to promote progression and metastasis of mammary tumours. Human breast tumours exhibit increased p-Tyr-lipin-1 levels compared to the adjacent tissues. Importantly, statistical analyses show that levels of p-Tyr-lipin-1 correlate with tumour sizes, lymph node metastasis, time to recurrence and survival of the patients. These results illustrate a direct lipogenesis-promoting role of the pro-oncogenic Src, providing a mechanistic link between obesity-associated mitogenic signaling and breast cancer malignancy.
The cellular proto-oncogene Src (c-Src) is a non-receptor tyrosine kinase, regulating various cellular processes, including those involved in cell proliferation, differentiation, survival and migration\(^1\). aberrantly activated Src has been known to be a potent oncogenic protein\(^2\). It is normally maintained in a catalytically inactive conformation by the intramolecular interaction of its carboxyl-terminal phosphotyrosine (Tyr530) with the N-terminal Src homology 2 (SH2) domain\(^3\). Engagement of receptor tyrosine kinases (RTKs) with pro-mitogenic growth factors such as EGF and PDGF leads to the dephosphorylation of Y530 and consequential de-inhibition of Src\(^4\). Activated Src then autophosphorylates tyrosine 416 residue (Tyr416, Tyr419 of human Src) in the kinase domain, enabling it to target a variety of substrates\(^5\). Mutations at the Y416 residue or within encompassing segments have been known to cause constitutive activation.

Cancer cells exhibit not only abnormally high demand for glucose and glutamine\(^6\) but also altered lipid metabolism such as elevated lipogenesis, increased fatty acid uptake. Lipid metabolism has an especially high impact on breast cancer cells\(^7\),\(^8\),\(^9\), as can be partitioned into the synthesis of TAGs or glycerophospholipogenesis. As a metabolic enzyme for glycerolipid synthesis, lipin-1 has an especially high impact on breast cancer cells\(^7\),\(^8\),\(^9\),\(^10\),\(^11\),\(^12\),\(^13\),\(^14\),\(^15\),\(^16\),\(^17\),\(^18\),\(^19\),\(^20\),\(^21\). Lipid metabolism is an important feature of cancer cells, where these cancer cells are often surrounded by a large number of adipocytes that actively undergo triglyceride (TAG) cycle and generate a fatty acid-rich environment. Fatty acids are consumed for energy production through β-oxidation, provide building blocks for phospholipids and act as extrinsic stimuli for cellular growth as well\(^9\),\(^10\),\(^11\). Phosphatidic acid phosphatase LPIN1 (lipin-1) possesses a dual function as a metabolic enzyme and a transcriptional cofactor for master regulators of lipid metabolism, including peroxisome proliferator-activated receptor α (PPARα)\(^12\),\(^13\) and sterol regulatory element-binding proteins (SREBPs)\(^14\), which in turn regulate other metabolic pathways such as fatty acid oxidation and de novo lipogenesis. As a metabolic enzyme for glycerolipid synthesis, lipin-1 catalyses the reaction of removing the phosphate group from phosphatidic acids (PA) to yield diacylglycerols (DAG) that in turn can be partitioned into the synthesis of TAGs or glycerophospholipids depending on the downstream enzymes\(^15\),\(^16\). Regulatory mechanisms have been identified for the posttranslational modifications of lipin-1, including phosphorylation\(^17\) and acetylation\(^18\). Lipin-1 has been found to be aberrantly upregulated in certain types of cancer cells, and its PAP activity is required for the survival of these cells\(^19\),\(^20\),\(^21\). However, the epistatic interactions between Src and lipin-1, as well as the functional linkage between oncogenic signalling and glycerolipid synthesis in vivo, remain obscure. In this study, through screening for lipin-1-interacting proteins, we found that the Src proto-oncogene protein interacts with and phosphorylates lipin-1. We have demonstrated that the PAP activity of lipin-1 is greatly increased after tyrosine phosphorylation by Src. We have provided evidence that pro-mitogenic growth factors signal to lipin-1 in an Src-dependent manner. Moreover, unphosphorylatable lipin-1 is unable to promote growth and metastasis of breast cancer spontaneously developed in PyVT/Lpin1\(^{−/−}\) mice in vivo. Our findings thus reveal that upregulating glycerolipid synthesis is an integral part of the tumour-promoting ability of Src, directly linking lipogenesis to tumour malignancy.

**Results**

**Src phosphorylates lipin-1 upon mitogenic stimulation.** To identify potential lipin-1 interacting proteins, we first replaced the endogenous lipin-1 with Flag-tagged counterpart in the MDA-MB-231 cell line of breast cancer origin by using the CRISPR/Cas9 technique (Supplementary Fig. 1a, b). The Flag-tagged lipin-1 was immunoprecipitated, followed by mass spectrometry analysis. Among the co-immunoprecipitated proteins, Src protein was identified as a potential new lipin-1-associated protein (Supplementary Fig. 2a, b). The interaction was verified, showing that the endogenous Src was co-precipitated with lipin-1 in wild-type MDA-MB-231 cells, but not in LPINI-knockout cells (Fig. 1a). We also performed co-immunoprecipitation in HEK293T cells transfected with Src and lipin-1 (Supplementary Fig. 2c, d). In vitro GST pull-down assay indicated a direct interaction between lipin-1 and Src (Fig. 1b and Supplementary Fig. 2e). Intriguingly, the interaction was diminished by Src inhibitors, SKI-606 and Dasatinib (Supplementary Fig. 2f, g); KD-Src (kinase-dead) and DN-Src (dominant-negative) mutants showed much-compromised interaction with lipin-1 (Supplementary Fig. 2h). Conversely, markedly higher levels of lipin-1 were co-precipitated with a constitutively active form of Src (YS29F-Src) (Supplementary Fig. 2h). These data indicated that an active structure or conformation of the Src kinase appears to be essential for its interaction with lipin-1.

We then asked whether lipin-1 is a direct phosphorylation substrate of Src. Enzymes involved in the TAG synthesis pathway were co-transfected with Src into HEK293T cells and were then immunoprecipitated (Supplementary Fig. 3a). As shown in Supplementary Fig. 3b, only lipin-1, but not the other enzymes, was tyrosine phosphorylated. After treatment with calf-intestinal alkaline phosphatase (CIP), the phosphorylated tyrosine (p-Tyr) signal disappeared (Supplementary Fig. 3c). In vitro kinase assays confirmed that Src could directly phosphorylate lipin-1 (Fig. 1c).

We thus examined whether growth factors would increase Src-mediated lipin-1 tyrosine phosphorylation by immunoblotting with a general antibody against phosphotyrosine after immunoprecipitation of lipin-1. In serum-deprived MDA-MB-231 cells treated with or without serum and growth factor (EGF), lipin-1 tyrosine phosphorylation was markedly increased, concurring with elevated phosphorylation of Src on Tyr416, which is known to be an activating modification for Src\(^1\) (Fig. 1d). Similar results were obtained when breast cancer cells were treated with EGF, PDGF and IGF-1 (Supplementary Fig. 3d–h). EGF treatment failed to increase the levels of p-Tyr-lipin-1 in SRC-knockdown MDA-MB-231 or MDA-MB-468 cells (Fig. 1e and Supplementary Fig. 3i). In addition, treatment of cells with the Src inhibitor Dasatinib abrogated EGF-increased lipin-1 phosphorylation (Supplementary Fig. 3j). Furthermore, reconstitution of SRC-KO cells with WT-Src, but not KD-Src, restored the EGF-increased tyrosine phosphorylation of lipin-1 (Fig. 1f and Supplementary Fig. 3k, l). These results illustrated that signalling from growth factors to Src can stimulate tyrosine phosphorylation on lipin-1.

**Tyrosine phosphorylation of lipin-1 promotes glycerolipid synthesis.** To identify the phosphorylated tyrosine residues, lipin-1 phosphorylated in vitro by Src was subjected to mass spectrometry. Multiple phosphorylated candidate tyrosine residues of lipin-1 were identified (Supplementary Fig. 4a, b). We then created mutants carrying alteration of those tyrosine residues to phenylalanine singly or in combination. It was found that the combined mutation of Tyr398, Tyr413 and Tyr795 (3YF) on lipin-1 strongly abolished Src-catalysed p-Tyr signal detected in lipin-1 immunoprecipitates (Fig. 2a–c). Sequence alignment indicates that these tyrosine residues in lipin-1 are highly conserved across different species (Fig. 2d and Supplementary Fig. 4c). We then raised polyclonal antibodies that specifically recognise lipin-1 phosphorylated at each of the three different tyrosine residues. By using these antibodies, we verified that the three tyrosine residues of lipin-1 are indeed phosphorylated in an Src-dependent manner in culture cells and in vitro (Fig. 2a, b). Moreover, compared to WT-lipin-1, the unphosphorylatable...
3YF-lipin-1 reintroduced to LPIN1-KO or -KD (knockdown) cells showed little, if any, p-Tyr signal even after growth factor treatment (Fig. 2c and Supplementary Fig. 4d, e). These data clearly demonstrated that lipin-1 is a bona fide substrate of Src. Considering the potential functional similarity of Src-family kinases, we also examined the effect of lipin-1 on three other broadly expressed Src-family members, Yes1, Fyn and Lyn. We found that Fyn and Lyn could also phosphorylate lipin-1 albeit at much lower efficiency, while Yes1 is unable to phosphorylate lipin-1 (Supplementary Fig. 4f).

It has been known that mTORC1 inhibits the PAP activity of lipin-1 via serine/threonine phosphorylation. We thus tested whether mTORC1-mediated phosphorylation would affect Src-mediated tyrosine phosphorylation of lipin-1 or vice versa. Under insulin- or EGF-stimulated conditions, Src-mediated tyrosine phosphorylation of lipin-1 was not affected by the mTOR inhibitor rapamycin or Torin 1, although mTOR inhibitors effectively blocked lipin-1 serine/threonine phosphorylation (Supplementary Fig. 5a–f). Similarly, the Src inhibitors did not affect mTORC1-mediated serine phosphorylation (Supplementary Fig. 5c–f), indicating that Src and mTORC1 regulate lipin-1 via independent mechanisms.

To find out the effect of Src-mediated tyrosine phosphorylation on lipin-1, we investigated the PAP activity of lipin-1 before and after phosphorylation by Src. WT-lipin-1 showed higher enzymatic activity than 3YF-lipin-1 in cells cultured in normal complete medium (Supplementary Fig. 6a). EGF treatment or Src overexpression increased the PAP activity of WT-lipin-1 (Fig. 2e and Supplementary Fig. 6a). By contrast, 3YF-lipin-1 failed to respond to such stimulation (Fig. 2e and Supplementary Fig. 6a). Of note, 3YE-lipin-1 and 3YD-lipin-1, putative p-Tyr mimetic mutants after changing Y to E or D, showed similar catalytic activity as 3YF-lipin-1, indicating that these substitutions cannot mimic Src-mediated tyrosine phosphorylation in lipin-1 (Supplementary Fig. 6a). We then determined the Michaelis constant (K_m) values of lipin-1, and found that Src-mediated phosphorylation benefits lipin-1 PAP activity likely by increasing the binding affinity of lipin-1 with its substrate PA (Supplementary Fig. 6b). It was reported that, in response to fatty acid, lipin-1 becomes serine/threonine dephosphorylated and translocates from cytoplasm to endoplasmic reticulum (ER)-associated membranes where the glycerolipid biosynthesis takes place. We thus examined whether fatty acid-induced ER localisation of lipin-1 is affected after tyrosine phosphorylation by Src. It was found that fatty acid-induced ER translocation of lipin-1 was not regulated by Src, indicating that the lipin-1 translocation is not influenced by tyrosine phosphorylation (Fig. 2f and Supplementary Fig. 6c, d), but likely through its acetylation as shown previously. Collectively, these results suggest that Src-mediated tyrosine phosphorylation of lipin-1 mainly upregulates the PAP activity. Consistent with the proteomic data showing that lipin-1 is the major PAP in human breast cancer samples, knockdown of LPIN1, but not its homologues LPIN2 or LPIN3, virtually abolished the PAP activity in breast cancer cell lines (Supplementary Fig. 6e, f), confirming that lipin-1 accounts for the majority of PAP activity in these cells.
We then investigated whether lipin-1 tyrosine phosphorylation by Src directly affects glycerolipid synthesis in MDA-MB-231 and non-malignant R. norvegicus 41E1 cells when normalised to protein abundance (Fig. 2g, h and Supplementary Fig. 7a, b). The mean values in the synthesis rate of phosphatidylcholine (PC) were reproducibly increased as well, while the level of phosphatidylinositol (PI) was decreased albeit with a lack of statistical significance (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b). The EGF-induced glycerolipid synthesis was markedly suppressed by the Src inhibitor Dasatinib (likely due to PA being driven to DAG as diagramed in Fig. 2g), while the level of phosphatidylinositol (PI) was decreased significantly (Fig. 2h and Supplementary Fig. 7a, b).
mediate the induction by EGF of the synthesis of TAG and phospholipids (PE and PS) when reintroduced to LPIN1-KO MDA-MB-231 cells or LPIN1-KD MDA-MB-468 cells (Fig. 2h and Supplementary Fig. 7b). The dependence of glycolipid synthesis on Src and tyrosine phosphorylation of lipin-1 was also demonstrated in cells cultured in normal complete medium (Supplementary Fig. 7e, f). Specifically, depletion of Src in WT-lipin-1 cells significantly impeded the synthesis of TAG, PE, PC and PS, while moderately increased PI synthesis (Supplementary Fig. 7g, h), and WT-lipin-1 but not the unphosphorylatable 3YF-lipin-1 increased the synthesis rates of these lipids (Fig. 2h and Supplementary Fig. 7b, e, f). In addition to the FAP activity, lipin-1 also possesses transcriptional coactivator activity and has been implicated in the regulation of fatty acid oxidation. By contrast, the regulatory role of lipin-1 in fatty acid oxidation was not affected by Src (Supplementary Fig. 7i).

**Proliferation of breast cancer cells depends on tyrosine phosphorylation of lipin-1.** To examine whether Src-accelerated glycolipid synthesis affects cell proliferation, we first knocked down **LPIN1** or SRC in MDA-MB-231 and MDA-MB-468 cells. It was found that reduction of lipin-1 or Src significantly impeded cell proliferation to similar extents, as indicated by CCK-8 and Fig. 7g, h), and WT-lipin-1 but not the unphosphorylatable 3YF-lipin-1 increased the synthesis rates of these lipids (Fig. 2h and Supplementary Fig. 7b, e, f). In addition to the FAP activity, lipin-1 also possesses transcriptional coactivator activity and has been implicated in the regulation of fatty acid oxidation. By contrast, the regulatory role of lipin-1 in fatty acid oxidation was not affected by Src (Supplementary Fig. 7i).

Tyrosine phosphorylation of lipin-1 in mouse mammary carcinoma. We next employed the mice expressing the Polyoma Virus middle T antigen under the direction of the mouse mammary tumour virus promoter/enhancer (MMTV-PyVT) to investigate the effect of lipin-1 in tumour development. These mice have been reported to show Src hyperactivity26,27. Along with the development of breast cancer, levels of Tyr-phosphorylated lipin-1 were increased in mammary tumours from the transgenic mice, congruent with elevated levels of the activated p-Tyr416-Src (Fig. 4a). To study for whether lipin-1 would affect the spontaneous development of mammary tumour, we crossed the MMTV-PyVT transgenic mice with Lpin1+/− mice, generating PyVT;Lpin1+/− mice. Deletion of Lpin1 had no effect on mammary gland morphogenesis as determined by carmine alum staining (Supplementary Fig. 9a). Meanwhile, the tumour frequency or tumour onset was also not significantly affected by lipin-1 deficiency (Supplementary Fig. 9b–d). However, PyVT;Lpin1+/− mice exhibited an obvious extension of overall survival time (Supplementary Fig. 9e–g). At 14 weeks of age, total tumour weights were significantly reduced in PyVT; Lpin1+/− mice, along with decreased actively proliferating cells, as indicated by Ki-67 staining (Fig. 4b and Supplementary Fig. 9h, i). Moreover, compared to PyVT;Lpin1+/− mice, much-increased numbers of the PyVT;Lpin1−/− mice either were free of, or contained fewer, tumour nodules metastasised to the lung surface (Fig. 4c and Supplementary Fig. 9j, k). However, it is formally possible that germline deletion of lipin-1 could also affect non-tumour cells, which in turn alter tumour metastasis. To clarify this, we transplanted WT and LPIN1-KO MMTV tumour cells separately into Lpin1+/+ or Lpin1−/− mice. While WT tumour cells exhibited stronger metastatic potential in Lpin1+/+ mice, Lpin1-KO tumour cells showed equally low metastasis in either Lpin1+/+ or Lpin1−/− mice (Supplementary Fig. 9l). Analysis of variance (ANOVA) reveals that tumour microenvironment that is determined by host genotypes accounts for 8.89% of the total variance in metastasis, while tumour cell-intrinsic difference that is determined by tumour genotypes accounts for 23.58% of the total variance in metastasis (Supplementary Fig. 9g). These data indicate that although lipin-1 has both cell-autonomous and microenvironment effects, with the former one playing a major part in determining tumour malignancy.

Given that the PyVT tumour model is known to mimic the Luminal B type breast cancer, we also tested the role of lipin-1 in different subtypes of breast cancer patient-derived xenografts (PDXs, Supplementary Data 2). It was found that lipin-1 knockdown in the PDX cells of Luminal A (ER+ HER2−), HER2+ and triple-negative breast cancer (TNBC) rendered these cells less metastatic after tail vein injection into the immunocompromised NOD-SCID female mice (Supplementary Fig. 9m–o), indicating that lipin-1 exerts a general pro-malignancy effect among different breast cancer subtypes.

To investigate the effect of tyrosine phosphorylation of lipin-1 on mouse mammary cancer, we first generated MMTV-Long Terminal Repeat (MMTV/LTR) promoter-driven adenov-associated viruses (AAV) carrying WT-lipin-1, 3YF-lipin-1 or GFP as a control, referred to as AAV-WT-lipin-1, AAV-3YF-lipin-1 and AAV-control. The mammary tissues of PyVT;Lpin1+/− mice were transduced with these AAVs before the onset of the tumour. It was found that the tumours were heavier in mice injected with AAV-WT-lipin-1 mice compared to AAV-3YF-lipin-1 or AAV-control mice, although tumour onsets were similar among these groups (Fig. 4d and Supplementary Fig. 9p, q). We next isolated and engineered PyVT;Lpin1+/− mice-derived spontaneous mammary tumour cells with or without lentivirus-mediated re-expression of WT-lipin-1 or 3YF-lipin-1. These cells were then employed in an experimental lung metastasis colonisation experiment by tail vein injection (Fig. 4e). Mice injected with tumour cells expressing 3YF-lipin-1 showed a much lower incidence of lung metastasis and reduced lung seeding compared to mice injected with WT-lipin-1 expressing or control tumour cells (Fig. 4f, g). These data indicated
Fig. 3 The proliferation of breast cancer cells depends on the tyrosine phosphorylation of lipin-1. 

a. Re-expression of WT-lipin-1 but not 3YF-lipin-1 restored the proliferation of breast cancer cells. LPIN1-KO MDA-MB-231 cells (a) or MDA-MB-468 cells knocked down of LPIN1 (LPIN1-KD) (b) infected with empty vector (ctrl), WT-lipin-1 or 3YF-lipin-1 and were maintained in complete medium containing 10% FBS. The CCK-8 assay (n = 4 experiments) and BrdU incorporation assay (n = 5 experiments) were performed to determine viable cell number. (Left graph of a) ctrl versus WT-lipin-1, \( P < 0.001 \) (day 3), \( **P < 0.001 \) (day 4); WT-lipin-1 versus 3YF-lipin-1, \( ###P < 0.001 \) (day 4). (Left graph of b) ctrl versus WT-lipin-1, \( **P = 0.0011 \) (day 4); WT-lipin-1 versus 3YF-lipin-1, \( ###P < 0.001 \) (day 4). c The soft agar colony formation assay was performed with LPIN1-KO MDA-MB-231 cells or LPIN1-KD MDA-MB-468 cells reconstituted with WT-lipin-1, 3YF-lipin-1 or empty vector as a control. The cells were maintained in complete medium containing 10% FBS. Scale bars, 5 mm. 

d. Xenograft tumour growth in mice. Volumes of tumour burdened in nude mice receiving LPIN1-KO MDA-MB-231 cells reconstituted with WT-lipin-1, 3YF-lipin-1 or empty vector as a control were measured on different days after implantation. n = 6 mice per group. Ctrl versus WT-lipin-1, \( *P = 0.0461 \) (day 19), \( *P = 0.0389 \) (day 22), \( *P = 0.0102 \) (day 25); WT-lipin-1 versus 3YF-lipin-1, \( P = 0.0161 \) (day 25). e Representative images and tumour weights of each independent experiment. 

f. TAG synthesis was performed in each independent experiment. 

Fig. 3 The proliferation of breast cancer cells depends on the tyrosine phosphorylation of lipin-1. a. Re-expression of WT-lipin-1 but not 3YF-lipin-1 restored the proliferation of breast cancer cells. LPIN1-KO MDA-MB-231 cells (a) or MDA-MB-468 cells knocked down of LPIN1 (LPIN1-KD) (b) infected with empty vector (ctrl), WT-lipin-1 or 3YF-lipin-1 and were maintained in complete medium containing 10% FBS. The CCK-8 assay (n = 4 experiments) and BrdU incorporation assay (n = 5 experiments) were performed to determine viable cell number. (Left graph of a) ctrl versus WT-lipin-1, \( P < 0.001 \) (day 3), \( **P < 0.001 \) (day 4); WT-lipin-1 versus 3YF-lipin-1, \( ###P < 0.001 \) (day 4). (Left graph of b) ctrl versus WT-lipin-1, \( **P = 0.0011 \) (day 4); WT-lipin-1 versus 3YF-lipin-1, \( ###P < 0.001 \) (day 4). c The soft agar colony formation assay was performed with LPIN1-KO MDA-MB-231 cells or LPIN1-KD MDA-MB-468 cells reconstituted with WT-lipin-1, 3YF-lipin-1 or empty vector as a control. The cells were maintained in complete medium containing 10% FBS. Scale bars, 5 mm. d. Xenograft tumour growth in mice. Volumes of tumour burdened in nude mice receiving LPIN1-KO MDA-MB-231 cells reconstituted with WT-lipin-1, 3YF-lipin-1 or empty vector as a control were measured on different days after implantation. n = 6 mice per group. Ctrl versus WT-lipin-1, \( *P = 0.0461 \) (day 19), \( *P = 0.0389 \) (day 22), \( *P = 0.0102 \) (day 25); WT-lipin-1 versus 3YF-lipin-1, \( P = 0.0161 \) (day 25). e Representative images and tumour weights of each independent experiment. 

f. TAG synthesis was performed in each independent experiment. 

g. Representative images and tumour weights of each independent experiment. 

h. Schematic diagram of the synthesis of PE and TAG from glycerol-3-phosphate (G-3-P) in mammalian cells. i Knockdown of EPT-1 or inhibition of TAG synthesis impeded the proliferation of breast cancer cells. MDA-MB-231 cells were knocked down of ethanolamine phosphotransferase-1 (EPT-1 KD) and/or treated with the DGAT inhibitors PF-04620110 (DGAT1 inhibitor) and PF-06424439 (DGAT2 inhibitor). The EPT-1 KD MDA-MB-231 cells were infected with HA-EPT-1, and were maintained in complete medium containing 10% FBS. The CCK-8 assays were performed to determine viable cell number. Ctrl shRNA versus DGAT1 + EPT-1 shRNA, \( P = 0.0236 \) (day 3), \( **P < 0.001 \) (day 4); ctrl shRNA versus EPT-1 shRNA, \( **P = 0.0027 \) (day 4); EPT-1 shRNA versus EPT-1 shRNA + HA-EPT-1, \( P = 0.0208 \) (day 4). a, b, i Quantified in each independent experiment. 

d-g. Quantified for each xenograft tumour. Data are mean ± s.e.m.; ordinary two-way ANOVA followed by Tukey in (left graph of a and b, d); ordinary one-way ANOVA followed by Tukey in (right graphs of a and b, e); two-way ANOVA (repeated measure) with Geisser-Greenhouse’s correction, followed by Tukey in d, two-tailed unpaired Student’s t-test in f, g. ***P < 0.001, N.S. not significant. Source data are provided as a Source Data file.
that tyrosine phosphorylation of lipin-1 is crucial for promoting the progression of mouse mammary tumour to malignancy.

Activation of the Src–lipin-1 axis and human breast cancer malignancy. We next analysed tyrosine phosphorylation of Src and lipin-1 in 44 human primary breast tumours that were instantaneously processed after surgery (Supplementary Data 3). A strong positive correlation between the signal of p-Tyr795-lipin-1 and p-Tyr416-Src was revealed (Fig. 5a–e and Supplementary Fig. 10a, b). P-Tyr795-lipin-1 and p-Tyr416-Src were markedly increased in human breast tumours compared to adjacent normal tissues (Fig. 5f, g). Importantly, p-Tyr795-lipin-1 was positively associated with tumour volume, with a significant linear trend identified across the levels of lymph-node metastasis and the clinical stages of the patients (Fig. 5h and Supplementary Fig. 10c, d). Moreover, we evaluated the correlation of p-Tyr795-lipin-1 with patient survival in a cohort of specimens from another 60 patients with invasive breast cancers for which the information of overall survival and relapse-free survival were available (Supplementary Data 4). Significant negative correlations of p-Tyr795-lipin-1 levels with overall survival and relapse-free survival were identified (Fig. 5i, j). A reduction of about 20 months in the median survival period was found in p-Tyr795-high patients, compared with those of low levels (Fig. 5k and Supplementary Fig. 10e, f). Strikingly, among patients who experienced cancer recurrence, the median time to recurrence of p-Tyr795-high patients was shortened to about 1/6 (upper quartile vs. lower quartile) or 1/3 (higher half vs. lower half) of the p-Tyr795-low patients (Fig. 5l and Supplementary Fig. 10g, h). These clinical associations demonstrate that the Src-mediated lipin-1 tyrosine phosphorylation is highly correlated with human breast cancer malignancy and poorer prognosis.

Discussion
Src is activated by a variety of extrinsic signals or by mutations in its upstream regulators such as EGFR. It has been reported that lipid droplets are positively correlated with Src levels in a panel of cancer cells. It has become increasingly clear that obesity influences the incidence and the mortality of a large variety of malignancies. Obese females were estimated to have more than 50% higher risk to develop breast cancer compared to those with normal BMI. Obese people tend to be associated with larger tumour size, increased lymph-node positivity, and lower survival. The association between obesity and cancer risk is particularly relevant to breast cancers as they are surrounded by adipose tissue. Excess adipose tissue is accompanied by a variety of local changes including altered lipid metabolism, hormone levels, inflammation, which form the microenvironment, as well as systemic changes that alter the physiology. Importantly, it was estimated that 40-70% of genetic factors that are related to cancer development were found to be strongly associated with obesity, including those involved in lipid metabolism, such as LIPE, hormone-sensitive lipase LIPE and fatty acid synthase FASN.
Because HER2 can decrease the rate of EGF dissociation from EGFR, leading to stronger and prolonged activation of EGFR, we consider EGF as a factor resembling the HER2 branch of Src-activation. By contrast, insulin and leptin that also activate Src are closely associated with obesity microenvironment (Supplementary Fig. 10i). Different from EGF, the effects of insulin and leptin in Src-activation are not apparently associated with HER2. Adipokines such as TNF-α and IL-6 have also been implicated in activating the Src-family kinases via toll-like receptors in various cell types. Moreover, the richness of fatty acids per se that are generally elevated in obesity in the microenvironment may contribute to tumour development. Fatty acid-binding proteins (FABPs) that function to solubilise various FAs and coordinate their trafficking inside the cell are recently reported as a new linkage between obesity and breast cancer. After entry into cancer cells, exogenous fatty acids cannot only serve as energy-producing nutrients but also act as secondary signalling agents. Therefore, their interaction with Src-family tyrosine kinases may contribute to the activation of these kinases. In this study, we found that the correlation between p-Tyr795-lipin-1 and p-Tyr416-Src in breast cancer is closely associated with Src-activation. In addition, inflammatory adipokines such as TNF-α and IL-6 have also been implicated in activating the Src-family kinases via toll-like receptors in various cell types. Moreover, the richness of fatty acids per se that are generally elevated in obesity in the microenvironment may contribute to tumour development. Fatty acid-binding proteins (FABPs) that function to solubilise various FAs and coordinate their trafficking inside the cell are recently reported as a new linkage between obesity and breast cancer. After entry into cancer cells, exogenous fatty acids cannot only serve as energy-producing nutrients but also act as secondary signalling agents. Therefore, their interaction with Src-family tyrosine kinases may contribute to the activation of these kinases.
messenger to coordinate signal transduction cascades that modulate carcinogenic processes. For example, saturated fatty acids alter the membrane distribution of Src, causing it to partition into intracellular membrane subdomains, where it becomes activated. Increased lipogenesis mediated by Src may be particularly relevant to breast cancer. According to Catalogue of Somatic Mutations in Cancer (COSMIC), Src is upregulated in 18.8% breast cancer cases, the highest upregulation rate among all Src-family members. Although point mutations and gene amplification of Src alone are not frequent, the total alteration rates among all the Src-family members combined (most of which are cases of gene amplification) are detected in 13% of the breast cancer patients, whose overall survival is shorter than patients without these changes (eBiportal for Cancer Genomics).

In line with these findings, we showed that the activating phosphorylation of Src and Src-mediated tyrosine phosphorylation of lipin-1 are markedly increased in human breast tumours compared to adjacent normal tissues. Moreover, we also identified a strong correlation of the activation of the Src–lipin-1 axis with tumour malignancy and poor prognosis. Of note, we found that unlike Src, Yes1 does not phosphorylate lipin-1, suggesting that Src-family members were not functionally redundant, which is consistent with a previous study showing different effects of c-Src- or c-Yes-deficiency on mouse mammary tumour.

In this study, we demonstrate the tumour intrinsic effect of lipin-1 in causing breast cancer malignancy through promoting lipid synthesis. However, one question remains in this study is whether lipin-1 in non-tumour cells also functions to shape pro-metastatic microenvironment. We show that the metastatic potential of WT MMTV tumour cells is reduced in Lipin-1−/− mice compared to Lipin-1+/- mice, and the non-tumour effect of lipin-1 contributes to 8.89% of the total variance in metastasis. It is conceivable that the presence or absence of lipin-1 in the neighbouring non-tumour cells would give rise to different compositions that would, in turn, alter contact between cancer and non-cancer cells or the contact between cancer cells and extracellular matrix.

In sum, it is remarkable that the proto-oncogene Src, apart from stimulating pro-mitogenic signalling cascades to cause tumours, plays a direct role in reprogrammed lipid metabolism, which endows cells with advantages in proliferation and metastasis.

Methods
Generation of CRISPR/Cas9-mediated knockout cell lines. Human cdon-optimised Cas9 (hCas9) and GFP-targeting gRNA-expressing plasmids were purchased from Addgene. The GFP-targeting sequence in the gRNA vector was replaced with the sequence 5′-AATTACGTTGGGAGGTATACG-3′ targeting exon 3 of human LPIN1 or 5′-GCCGCGGCTGTCGCAGTCTTT-3′ targeting exon 4 of human SRC. To construct the knockout cell lines, MDA-MB-231 cells were transfected with 1.5 μg of a gRNA-expressing plasmid and 1.5 μg of hCas9 plasmid and subjected to blasticidin selection for 3 days. The resultant resistant cells were then sorted into single clones in the 96-well plate. Single clones were screened by genomic PCR with primers targeting the upstream region of the gRNA (5′-CGCG AAATGTTTGGAACG-3′ for human LPIN1 or 5′-GTGTCTGAGCTAACTGCTGCTG-3′ for human SRC) and the downstream region of the gRNA (5′-CAAGACAGGTCCATCAACAGGATCC-3′ for human LPIN1 or 5′-GAATGAGGGACCTTCCG AA CAG-3′ for human SRC). Positive clones were further confirmed by immunoblotting. The sequences of the primers are also shown in Supplementary Data 5.

Protein analysis by mass spectrometry. To identify the lipin-1 interacting proteins, Flag-tagged lipin-1 was expressed in LPIN1-KO MDA-MB-231 cells, immunoprecipitated with antibody to Flag, and subjected to SDS-PAGE, followed by silver staining. Gels were excised and digested by trypsin, followed by mass spectrometry analysis. To identify the tyrosine phosphorylation sites on lipin-1, HEK293T cells were transfected with Flag-lipin-1 with HA-Src or empty vector as a control. Flag-lipin-1 was then immunoprecipitated with anti-Flag antibody and subjected to SDS-PAGE, followed by silver staining. Gels were excised and digested by trypsin, followed by mass spectrometry analysis.

Antibodies and reagents. Antibodies for phospho-Ser106-LPIN1 (1:1000 for IB) were described previously. The rabbit polyclonal antibodies that specifically recognize phosphorylated lipin-1 were generated by immunizing rabbits with synthetic peptides as follows: DKKRSHILAGDV(pTyr)110 for phospho-Tyr98-LPIN1 (1:500 for IB), EVAAL(pTyr)FPKNGDSPS for phospho-LPIN1 (1:500 for IB), and TEP(pTyr)AAGFNPADPV for phospho-Tyr305-lipin-1 (1:500 for IB, 1:50 for IHC). Antibodies to phospho-LPINS-5′-3′ (1:100, Cat. 6921, Src, 1:100, Cat. 2123, lipin-1 (1:1400), phospho-Tyr41 (1:9496), phospho-LPIN2 (1:1000, Cat. 2128), phospho-p70 S6 Kinase (1:1000, Cat. 9205), S70 S6 Kinase (1:1000, Cat. 9202) and Ki-67 (1:400 for IHC, Cat. 12202) were purchased from Cell Signaling Technology. Antibodies to lipin-1 (1:1000 for IB, H-120; 1:50 for IF, B-12, HA) (1:1000, Cat. 2123), anti-normal rabbit IgG (1:5000, Cat. 2120, B2) and c-Src (1:2000, Cat. 2120) were obtained from Santa Cruz Biotechnology. Antibodies to Flag (1:5000, Cat. F2555 and SAB420071) and Actin (1:5000, Cat. A1978) were purchased from Sigma. Anti-Calnexin (1:1000 for IB, 1:1000 for IF, Cat. 10427-2-AP) and anti-GST (1:5000, Cat. 66601-2-1g) antibodies were purchased from Proteintech. Anti-LPINS1 (1:500, Cat. LS-C339442) was from LSbio. Anti-SELDI (1:1000, EPT-1, Cat. H90080465-A01 was from Novus Biologicals. Donkey anti-rabbit IgG secondary antibody Alexa Fluor 488 (Diluted 1:100 in PBS, Cat. A21206), and donkey anti-mouse IgG secondary antibody Alexa Fluor 594 (Diluted 1:100 in PBS, Cat. A21203) were purchased from ThermoFisher Scientific. The HRP-conjugated goat anti-Mouse IgG (1:150-005-003, 1:5000 for IB) antibodies were purchased from Jackson ImmunoResearch. Anti-Flag beads (M-) (Cat. M8823) and oleic acid (Cat. O1383) were purchased from Sigma. Rapamycin (Cat. S1039), Torin 1 (Cat. S287), Blastidicin (Cat. S7419), Src inhibitors Dasatinib (Cat. S0121) and SKI-606 (Cat. S0114) were purchased from Selleckchem. Recombinant human EGF (Cat. 236-EG), human PDGF-BB (Cat. 220-BB) and human PDGF-AA (Cat. 291-G1) were purchased from PeproTech. Laminin-1 (10 g/ml) and Methocel K4M (4%) were purchased from BD Biosciences. Polyethyleneimine (Polyscience, Cat. 23966) at a concentration of 1 mg/ml was used to express control shRNA (5′-ATTACGTGGGGCAGTTAGC-3′) or c-Src shRNA. The sequences of the primers are also shown in Supplementary Data 5.

RNA interference and lentivirus-mediated infection. The lentivirus vector pLl3.7 was used for expression of shRNAs in cells. The 19-nucleotide sequence for each shRNA is as follows: 5′-GCTCCAGATTTGCGAACAC-3′ (si1) and 5′-GGAC CTGTCGTCGGGCAGA-3′ (si2) for human SRC. 5′-GGATGGACATCATGT GCTGTA-3′ for human Lpin1, 5′-GGAGACAGCCCAAGGGAGCAT-3′ for mouse Lpin1, 5′-GGGAGTGTTGCAAGTGTTAGT-3′ for human LPIN2, 5′-GGCTTCTCTCCGAGT ACAGAT-3′ for human LPIN3, 5′-GCTGCTGTGCTGTTGCTTAGT-3′ for human EPT1. plL3.7-Benilis was used to express control shRNA (5′-TATGGCGGG TGATTTATAC-3′). The sequences of the primers are also shown in Supplementary Data 5. Lentiviruses for infection were packaged in HEK293T cells using a liposomal transfection reagent (YEASEN, Cat. 40802E503). Viruses were collected 48–72 h after transfection and stored at −80°C until use. MDA-MB-231, MDA-MB-468 or MCF-7 cells were maintained in McCoy’s 5a medium ( Gibco) supplemented with 10% FBS and P/S. MDA-MB-231 and MDA-MB-468 cells were kept in L-15 medium (Gibco) supplemented with 10% FBS and P/S. All cell lines were validated to be free of mycoplasma contamination. Polyethyleneimine (Polyscience, Cat. 23966) at a final concentration of 10 μg/ml was used for transfection of HEK293T cells. Cells were harvested 20–24 h after transfection with a lysis buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mg/μl Triton, 2.5 mM sodium pyrophosphate, 1 mM β-mercaptoethanol, 2 μg/ml leupeptin and 1 mM PMSF] and subjected to immunoprecipitation.
Immunoprecipitation and immunoblotting. Cells for immunoprecipitation were lysed on ice using lysis buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% (v/v) FNE, 2.5 mM sodium ortho-phenylphosphorylacetate, 1 mM Na3VO4, 2 µg/ml leupeptin and 1 mM PMSF], and subjected to immunoprecipitation using antibodies as indicated. The immunoprecipitates were then washed three times with lysis buffer and solubilized in SDS buffer for immunoblotting. The total cell lysates and immunoprecipitates were boiled separately and subjected to 10% SDS-PAGE. Primary antibodies were used according to manufacturers’ protocols. Proteins were visualised by enhanced chemiluminescence using horseradish peroxidase-conjugated antibodies.

**Protein purification and in vitro GST pull-down assay.** Full-length cDNAs of lipin-1, non-phosphorylatable mutants of lipin-1 (3YF-lipin-1), Src and a kinase-dead form of Src (KD-Src) were cloned into pET-28a or pGEX 4T-1 vector. Protein expression was performed in the strain E. coli BL21 strain. The transformed E. coli cells were incubated for 2 h with 1 mM isopropyl-β-D-thiogalactopyranoside to induce the expression of His-tagged lipin-1, Src and KD-Src or GST-tagged Src. Ni2+-NTA-agarose (Sigma, Cat. P6611) was used for the purification of His-tagged proteins and glutathione sepharose beads (GE Healthcare, Cat. 17513202) were used for the purification of GST-tagged Src. In pull-down assay, the bound GST or GST-Src proteins were incubated with purified His-lipin-1, GST or GST-Src. Pull-down buffer [150 mM NaCl, 50 mM Tris-HCl (pH 7.6), 0.5% Nonidet P-40, and 1 mM PMSF] at 4 °C for 3 h. The precipitates were washed for 3 times with pull-down buffer and visualised by ponceau brilliant blue staining. Immunoblot was also carried out using anti-lipin-1 and anti-GST antibodies to detect His-lipin-1, GST or GST-Src.

**In vitro kinase assay.** In vitro kinase assay was performed based on a previously described protocol35. In brief, purified bacterially expressed His-tagged WT-lipin-1 or 3YF-lipin-1 and purified His-tagged Src or WT-Src were incubated in a kinase buffer [0.5% DMSO, 5 mM MgCl2, 1 mM DTT, 0.5 mg/ml BSA, 1 mM Na3VO4, 3 μCi 32P-labelled ATP and 0.5 μCi [γ-32P]-ATP (PerkinElmer, Cat. NEG5222S0U(0)C) for 30 min at 28 °C. Reactions were stopped by addition of SDS sample buffer, followed by SDS-PAGE, transferring to PVDF membrane and autoradiography.

**PAP activity measurement.** For the PAP activity measurement of ectopically expressed lipin-1, Flag-tagged WT-lipin-1, 3YF-lipin-1, 3YE-lipin-1 and 3YF-lipin-1 with or without HA-tagged Src were transfected into HEK293T cells. Cells were lysed 24 h after transfection. Flag antibody-conjugated beads (Sigma, Cat. M8823) were then used for immunoprecipitation of lipin-1 for 3 h at 4 °C. The immunoprecipitates were then washed three times with lysis buffer and twice with 25 mM Tris-HCl (pH 7.5) buffer. The Flag-tagged proteins were then eluted with 3 μl Flag peptide (Sigma, Cat. F3290) in 25 mM Tris-HCl (pH 7.5) buffer containing 150 mM NaCl and protease inhibitors. For measurement of the PAP activity of endogenous lipin-1, MBA-MB-231 or MBA-MB-468 cells were seeded in 100-mm dishes and grown in complete L-15 medium for 12–16 h. Then, cells were incubated with L-15 medium with or without serum for 4 h and then treated with or without serum or EG.F. Cells were then washed once with ice-cold PBS, harvested with Triton X-100 lysis buffer and followed by determination of PAP activity.

The PAP assay was carried out based on a previously described protocol34. The PAP activity was measured in a reaction buffer [0.1 mM Tris/maleate (pH 6.9), 150 mM NaCl and protease inhibitors]. For measurement of the PAP activity of endogenous lipin-1, MBA-MB-231 or MBA-MB-468 cells were seeded in 100-mm dishes and grown in complete L-15 medium for 12–16 h. Then, cells were incubated with L-15 medium with or without serum for 4 h and then treated with or without serum or EG.F. Cells were then washed once with ice-cold PBS, harvested with Triton X-100 lysis buffer and followed by determination of PAP activity.

**Glycerol synthesis assay.** MBA-MB-231 and MBA-MB-468 cells were treated with 0.25 mM BSA-bound oleic acid mixed with 3 μCi [9,10-3H]-oleic acid and 0.8 μCi [1,2-14C]-oleic acid for 1 h at 37 °C. 480 μl of supernatant was then transferred and mixed with 192 μl of 1.3 M perchloric acid, followed by centrifugation at 10,000 x g for 1 min to remove precipitated proteins. 500 μl supernatant was then mixed with 4 ml of scintillation liquid to determine the 3H radioactivity. Cell-free samples were also used for each experiment to ensure that no more than 20% of labelled oleic acid was oxidised during the assay. The fatty acids were β-oxidised to produce 14CO2, the amounts of which were determined by a scintillation counter. PAP activity was normalized to the amount of 18S ribosomal RNA for each sample by Bradford assay. The amount of 3H2O generated was expressed as DPM fatty acid per mg protein per hour.

**Immunofluorescence.** Immunofluorescence was performed based on a previously described protocol35. In brief, MBA-MB-231 cells were plated on glass coverslips in 12-well plates at 30–40% of confluence. Cells were treated with BSA or OA 2 h before fixation. After fixation in 4% paraformaldehyde, the cells were rinsed with PBS, permeabilized with 0.5% saponin in PBS for 10 min, and then blocked with 5% normal donkey serum for 1 h at room temperature. The coverslips were incubated with antibodies to lipin-1 and Calnexin overnight at 4 °C. Alexa-Flour 488-conjugated and 594-conjugated secondary antibodies were used to label the target protein. Fluorescent images were captured using a Zeiss Laser Scanning Confocal microscope. Quantification was performed by using the JACoP plugin for Image J.

**Cell proliferation and anchorage-independent growth assay.** Cell counting kit-8 (CCK-8) (MCE, Cat. HY-K0301) and cell proliferation ELISA, BrdU (colori- metric) (Roche, Cat. 11647229001) were used to determine cell proliferation according to manufacturer’s instructions. For anchorage-independent growth assay, cells were cultured in 6-well plates at 37 °C. After 3 days, colonies were fixed in 4% paraformaldehyde, the cells were stained with 0.5% crystal violet in methanol and then washed with water. Colonies were counted with an epifluorescence microscope. All animal procedures were performed with an approved protocol from the Institutional Animal Care and Use Committee at Xiamen University. Mice were housed in a temperature-controlled environment under a 12 h light/dark cycle with free access to water and standard rodent chow diet at 23 ± 3 °C and 30% humidity. FVB/N-Tg (MTMV-PyVT) 634Mul/J (PyVT, Stock No. 002374) and BALB/cByJ mice were used for immunoprecipitation of lipin-1 for 3 h at 4 °C. Alexa-Flour 488-conjugated and 594-conjugated secondary antibodies were used to label the target protein. Fluorescent images were captured using a Zeiss Laser Scanning Confocal microscope. Quantification was performed by using the JACoP plugin for Image J.
were orthotopically injected into the fourth mammary fat pad of 8-week-old female nude mice or FVB/N female mice. One week post-injection, tumour volumes were measured using a caliper (cm², Acculine, Solven) on a minimum of three euthanized and xenograft tumours were dissected for analysis. For lung metastasis colonisation model, PyVT/Lpin1+/− mice-derived tumour cells (1 x 10^6) were injected through the tail vein of 6-week-old FVB/N female mice; 1 x 10⁵ WT or Lipin-1 KO MMTV cells were injected intravenously into either six-week-old Lpin1+/− or Lpin1−/− female mice; the patient-derived xenograft (PDX)–derived Linalum A (ERβ2−/+). triple-negative breast cancer (TNBC) or HER2 positive (HER2+) breast cancer cells expressing shRNA targeting LPIN1 (LPIN1 shRNA) or Renilla as a control (ctrl shRNA) were injected through the tail vein of six-week-old immunocompromised NOD-SCID female mice.

**Carmine alun staining of the mammary fat pad.** The fourth mammary fat pads were removed from Lpin1+/+ and Lpin1−/− mice and quickly spread onto ice-cold glass slides, and then fixed in Carnoy’s fixative (ethanol:chloroform:glacial acetic acid = 6:3:1, v/v/v) for 2–4 h. The glands were then washed with 70% ethanol for 15 min, rinsed in distilled water once for 5 min, and stained with Carmine Alum (Sem Cell Technologies, Cat. 07070) according to the manufacturer’s instructions. The slides were washed with ethanol (70%, 95% and 100%), cleared with xylene and mounted with neutral balsam. Images were taken an Epson Expression 11000XL Graphics Arts scanner.

**Immunohistochemistry.** Sections of 5 μm were baked 4 h at 75 °C, followed by deparaffination with xylene and a graded series of ethanol. The sections were then washed with PBS, boiled in 10 mM sodium citrate buffer (pH 6.0) at 100 °C for 30 min. After rinsing twice in PBS, sections were incubated with 3% H₂O₂ for 30 min to block endogenous peroxidase, blocked at room temperature for 1 h by using 1% BSA, followed by incubation with p-Y795-lipin-1 or Ki-67 antibody (dilution 1:100). Following PBS washes, sections were then incubated with HRP-conjugated goat anti-rabbit or mouse secondary antibody for 1 h at room temperature. Sections were stained by DAB Peroxidase (HRP) Substrate Kit (Vector Laboratories, Cat. SK-4100) and then counterstained with hematoxylin according to manufacturer’s protocols.

**Generation and delivery of recombinant adeno-associated viruses (AAV).** To generate AAV of serotype 9 (AAV-9), HEK293T cells were transfected with AAV transfer plasmid containing sequence encoding WT-lipin-1, 3YF-lipin-1 or GFP as a control, together with AAV-9 helper plasmids. After 60–72 h of transfection, cells were harvested and resuspended with buffer A (50 mM Tris-HCl (pH 8.0) and 150 mM NaCl). The crude virus was released from the cell suspension after three freeze/thaw cycles and purified with a graded series of Density Gradient Medium (Sigma, Cat. D1556). The virus fraction was dialysed in PBS and concentrated by Millipore Ultra centrifugal filter. AAV titre was determined by RT-PCR using primers targeting the MMTV-LTR promoter. The 6-week-old Lpin1−/− female mice were anaesthetised and injected with 1 x 10⁵ genome copies of AAV per fat pad, 6 weeks post-injection, tumour weights of these mice were measured.

**Clinical samples.** Human breast cancer samples were from Sun Yat-Sen University Cancer Center (Guangzhou, China) with written informed consent from the patients. All the procedures were approved by the Institutional Review Board of Sun Yat-Sen University Cancer Center (Guangzhou, China) and conducted in accordance with the Declaration of Helsinki. Breast tumour samples (44 tumour sample and 44 matching adjacent normal tissues) were obtained from patients and were quickly processed to ensure the quality of the clinical samples, and stored at −80 °C wherever necessary for further use. The clinical stages of breast cancer were classified according to the tumour-node-metastasis classification of the Sixth Edition of the AJCC Cancer Staging Manual.

The breast cancer tissue microarray (60 cases) was prepared at the Xijing Hospital, Fourth Military Medical University, Shaanxi, China. The immunohistochemistry data were quantified using the immunoreactive score (IRS) system28. Each sample was evaluated by 3 persons individually in a blinded manner and the mean score was considered as the final IRS. Patients were grouped based on IRS scores of p-Y795-lipin-1 levels. The p-Y795-lipin-1 high- and low- groups were determined based on the median or quartiles calculated across the entire dataset.

**Lipid extraction and lipidomic analysis by LC-MS/MS.** Frozen tumour tissues were inactivated by addition of 1 ml mixture containing methanol, MTBE and internal standard mixture. Tissue samples were homogenised on an automated bead mixer, followed at 4 °C for 2 min. Then, 500 μl of deionized H₂O was added to the mixture, followed by centrifuged with 16,000 x g at 4 °C for 10 min. The extract supernatant was transferred to a new tube and dried. Samples were stored at −80 °C until mass spectrometric analysis. The lipidomic analysis by LC-MS/MS was performed by Metware Biotechnology Co., Ltd (Wuhan, China) based on a previously described protocol39, with slight modifications. In brief, the sample extracts were analysed using a LC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM A system, https://www.shimadzu.com/; MS, Q TRAP System, https://sciex.com/). The analytical conditions were as follows. UPLC: column [Thermo C30 (2.6 μm, 2.1 mm × 100 mm), solvent A: acetonitrile/water (60/40, v/v) containing 0.04% acetic acid and 5 mM ammonium formate, B: acetonitrile/isopropanol (10/90, v/v) containing 0.04% acetic acid and 5 mM ammonium formate], a gradient series of A/B programme [80:20 (v/v) at 0 min, 30:70 (v/v) at 3.5 min, 35:65 (v/v) at 5 min, 25:75 (v/v) at 9 min and 10:90 (v/v) at 15.5 min, flow rate (0.33 ml/min), temperature, 45 °C]. Injection volume: 2 μl. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS. LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), Q TRAP LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: an ion source, turbo spray; source temperature 550 °C; ion-spray voltage (IS) 5500 V; ion source gas I (GS1), gas II (GSII), curtain gas (CUR) were set at 35, 60 and 25 psi, respectively; the collision gas (CAD) was medium. Instrument tuning and mass calibration were performed prior to each run. Log-rank (Mantel–Cox) test was used to compare the survival distributions of the two groups. For other graphs showing representative data, reproducibility is stated as: (1) n ≥ 3 biologically independent experiments for Figs. 1a–f; 2a–c, f; 3a; 4a; Supplementary Figs. 1b; 2c–h; 3b–j; 4a–f; 5a–f, 6c, 7c, 8a, b, e (immuno blotting), i, k, o, v (immunoblotting); 9m–o (immunoblotting), p, (2) n = 3 technically independent experiments for Fig. 5a–d, Supplementary Figs. 10a, b. (3) n = 1 experiment for Supplementary Fig. 2a.

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

**Data availability.** The authors declare that all the data supporting the findings of this study are available within the paper and its supplementary information files. Primary antibodies and the nucleotide sequence for each shRNA used in this study are described in the ‘Methods’ section. Source data are provided with this paper.

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Author contributions

S.-C. L., S.-S. and S.-Y. L. conceived the project, designed the experiments, analysed data and wrote the paper. L.-S. H.-H., Y.-Y., T.-Y., J.-C., Y.-S., H.-Z. and L.C. performed experiments and participated in the design of the results. Z.-Z. L. and D.-T. L. assisted with animal experiments. Z.-Z. L. and Y.J. provided breast cancer patient samples. J.C. and E.S. provided the patient-derived xenograft (PDX)-derived cells. X.H. contributed to the generation of antibodies. C.X. contributed to the identification of lipin-1 phosphorylation sites by LC-MS/MS.

Competing interests

The authors declare no competing interests.
Additional information
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