ALTERATIONS IN BOTH CELL METABOLISM AND TRANSCRIPTIONAL PROGRAMS ARE HALLMARKS OF CANCER THAT SUSTAIN RAPID PROLIFERATION AND METASTASIS. HOWEVER, THE MECHANISMS THAT CONTROL THE INTERACTION BETWEEN METABOLIC REPROGRAMMING AND TRANSCRIPTIONAL REGULATION REMAIN UNCLEAR. HERE WE SHOW THAT THE METABOLIC ENZYME 6-PHOSPHOFRUCTO-2-KINASE/fructose-2,6-bisphosphatase 4 (PFKFB4) REGULATES TRANSCRIPTIONAL REPROGRAMMING BY ACTIVATING THE ONCOGENIC STEROID RECEPTOR COACTIVATOR-3 (SRC-3). WE USED A KINOME-WIDE RNA INTERFERENCE-BASED SCREENING METHOD TO IDENTIFY POTENTIAL KINASES THAT MODULATE THE INTRINSIC SRC-3 TRANSCRIPTIONAL RESPONSE. PFKFB4, A REGULATORY ENZYME THAT SYNTHESIZES A POTENT STIMULATOR OF GLYCOLYSIS, IS FOUND TO BE A ROBUST STIMULATOR OF SRC-3 THAT COREGULATES ESTROGEN RECEPTOR. PFKFB4 PHOSPHORYLATES SRC-3 AT SERINE 857 AND ENHANCES ITS TRANSCRIPTIONAL ACTIVITY, WHEREAS EITHER SUPPRESSION OF PFKFB4 OR ECTOPIC EXPRESSION OF A PHOSPHORYLATION-DEFICIENT SER857A ALA MUTANT SRC-3 ABOLISHES THE SRC-3-MEDIATED TRANSCRIPTIONAL OUTPUT. FUNCTIONALLY, PFKFB4-DRIVEN SRC-3 ACTIVATION DRIVES GLUCOSE FLOW TOWARDS THE PENTOSE PHOSPHATE PATHWAY AND ENABLES PURINE SYNTHESIS BY TRANSCRIPTIONALLY UPREGULATING THE EXPRESSION OF THE ENZYME TRANSKETOLASE. IN ADDITION, THE TWO ENZYMES ADENOSINE MONOPHOSPHATE DEAMINASE-1 (AMPD1) AND XANTHINE DEHYDROGENASE (XDH), WHICH ARE INVOLVED IN PURINE METABOLISM, WERE IDENTIFIED AS SRC-3 TARGETS THAT MAY OR MAY NOT BE DIRECTLY INVOLVED IN PURINE SYNTHESIS. MECHANISTICALLY, PHOSPHORYLATION OF SRC-3 AT SER857 INCREASES ITS INTERACTION WITH THE TRANSCRIPTION FACTOR ATF4 BY STABILIZING THE RECRUITMENT OF SRC-3 AND ATF4 TO TARGET GENE PROMOTERS. ABLATION OF SRC-3 OR PFKFB4 SUPPRESSES BREAST TUMOUR GROWTH IN MICE AND PREVENTS METASTASIS TO THE LUNG FROM AN ORTHOTOPIC SETTING, AS DOES SER857ALAMUTANT SRC-3. PFKFB4 AND PHOSPHORYLATED SRC-3 LEVELS ARE INCREASED AND CORRELATE IN OESTROGEN RECEPTOR-POSITIVE TUMOURS, WHEREAS, IN PATIENTS WITH THE BASAL SUBTYPE, PFKFB4 AND SRC-3 DRIVE A COMMON PROTEIN SIGNATURE THAT CORRELATES WITH THE POOR SURVIVAL OF PATIENTS WITH BREAST CANCER. THESE FINDINGS SUGGEST THAT THE WARBURG PATHWAY ENZYME PFKFB4 ACTS AS A MOLECULAR FULCRUM THAT COUPLES SUGAR METABOLISM TO TRANSCRIPTIONAL ACTIVATION BY STIMULATING SRC-3 TO PROMOTE AGGRESSIVE METASTATIC TUMOURS.

AMONG THE LANDSCAPE OF GENETIC ALTERATIONS THAT DRIVE AGGRESSIVE METASTATIC TUMOURS, THE TRANSCRIPTIONAL COREGULATOR SRC-3 IS ONE OF THE ABUNDANTLY Deregulated oncogenes. Importantly, dynamic interactions between SRC-3 and its subsequent recruitment to target genes are delicately regulated by post-translational modifications on SRC-3. Phosphorylation of SRC-3 can alter its transcriptional activity, protein stability and subcellular localization, and deregulated kinase signalling hyperactivating SRC-3 is a hallmark of many tumours. As a starting point for identifying kinases that modulate SRC-3 transcriptional activity, we performed an unbiased RNA interference (RNAi) screening assay using a kinome library containing short interfering RNAs (siRNAs) that target 636 human kinases (median 3 siRNAs per kinase) in the presence of a GAL4-DNA binding domain-fused SRC-3 (pBIND-SRC-3) and GAL4 DNA-binding sites containing the luciferase reporter gene (pG5-luc) (Fig. 1a). The concentration of the pBIND-SRC-3 construct needed to obtain luciferase readings in a linear range was standardized along with the dose of kinase siRNAs to observe significant alterations in SRC-3 intrinsic activity (Extended Data Fig. 1a, b). As a positive control, we used siRNAs that target PRKCZ1, a protein kinase known to activate SRC-3, and compared the repression of the coregulator activity after kinase knockdown with non-targeting control green fluorescent protein (GFP) siRNAs (Extended Data Fig. 1c). Kinome-wide screening identified several kinases as modulators of SRC-3 activity (Fig. 1b, Extended Data Fig. 1d and Supplementary Table 1), as either stimulators or repressors compared to the controls (Extended Data Fig. 1e).

Ten kinases were designated as reproducible and significant hits in the screen (Fig. 1c and Extended Data Fig. 1f), among which metabolic kinase PFKFB4 was identified as the most robust positive regulator of SRC-3 activity. A secondary screen coupled with growth assays to identify the top-hit kinases that drive cancer cell proliferation also identified PFKFB4 as the most dominant kinase that regulates cellular proliferation (Extended Data Fig. 1g). Silencing of PFKFB4 with different short hairpin RNAs (shRNAs) and siRNAs decreased SRC-3 activity (Extended Data Fig. 2a, b) in several cancer lines with reduced PFKFB4 levels (Extended Data Fig. 2c, d), whereas ectopic overexpression of PFKFB4 using adenoviral infection (ad-PFKFB4) enhanced SRC-3 activity (Fig. 1f). Interestingly, SRC-3 protein levels were increased after ectopic PFKFB4 expression (Fig. 1e), but SRC-3 (also known as NCOA3) mRNA levels were not affected (Extended Data Fig. 2e), and proximity ligation assays support a direct interaction between SRC-3 and PFKFB4, consistent with PFKFB4-dependent regulation of SRC-3 activity (Extended Data Fig. 2f).

PFKFB4 is a bifunctional metabolic enzyme that synthesizes fructose 2,6-bisphosphate (F2,6-BP), an important sugar-phosphate metabolite that stimulates glycolysis. PFKFB4 deters two antagonistic properties involving a kinase reaction synthesizing F2,6-BP from fructose-6-phosphate (F6P) and ATP, and conversely hydrolysing F2,6-BP into F6P and inorganic phosphate (Pi) via its phosphatase activity. These properties of PFKFB4 prompted us to investigate whether PFKFB4-catalysed enzymatic reactions could increase phosphorylation on SRC-3. In an in vitro enzymatic reaction containing F6P, ATP and varying concentrations of recombinant PFKFB4 enzyme were incubated with purified full-length SRC-3 protein. Increasing the amount of PFKFB4 enzyme in the reaction concomitantly enhanced the Ser/Thr phosphorylation of SRC-3, indicating that the metabolic enzyme PFKFB4 can phosphorylate a protein substrate (Extended Data Fig. 3a). We investigated the phosphate donor in the PFKFB4 kinase reaction, and identified ATP as being required for SRC-3 phosphorylation by PFKFB4 (Extended Data Fig. 3b). These findings suggest that PFKFB4 can function as a protein kinase to phosphorylate SRC-3 by transferring...
a phosphate group from ATP. To confirm this observation, we performed a kinase assay using [γ-32P]ATP as the phosphate donor and observed enhanced incorporation of phosphate from [γ-32P]ATP into SRC-3 protein upon increasing concentrations of the PFKFB4 kinase in the reaction (Fig. 2a). To identify the phosphorylation site(s) on SRC-3, we used recombinant glutathione S-transferase (GST)-fused SRC-3 fragments encoding various domains (Extended Data Fig. 3c) as substrates for an in vitro kinase reaction, and found that only the CBP-interacting domain (CID) of SRC-3 is phosphorylated by PFKFB4 (Fig. 2b). In vitro phosphorylated GST–SRC-3–CID protein was then analysed by mass spectrometry, and only one serine residue (Ser857) was identified as a phosphorylation target of PFKFB4 (Extended Data Fig. 3d). Consistent with this identification, mutation of Ser857 to alanine (Ser857Ala) abolished the phosphorylation of SRC-3–CID by PFKFB4 in vitro (Extended Data Fig. 3e), confirming that PFKFB4 phosphorylates oncopgenic coactivator SRC-3 at Ser857.

Because increased glucose metabolism stimulates the kinase activity of PFKFB4 required to maintain steady glycolysis, we measured the levels of phosphorylated SRC-3 (pSRC-3) under these conditions. HEK293T cells were transfected with Flag-tagged SRC-3 and PFKFB4, and then stimulated with an increasing concentration of glucose in culture medium, which revealed enhanced phosphorylation of SRC-3 (Fig. 2c). Next we investigated the levels of pSRC-3-S857 in breast cancer cells under conditions of active glycolysis by immunoblotting with a pSRC-3–Ser857-antibody. MDA-MB-231 cells growing under normal glucose condition (25 mM) showed robust phosphorylation of SRC-3 at Ser857 compared to tumour cells cultured in low glucose conditions (5 mM) (Fig. 2d). Withdrawing glucose from the medium after growth in normal glucose conditions (25 mM) resulted in significant loss of SRC-3 phosphorylation (Fig. 2d). Moreover, stable knock-down of PFKFB4 using two different shRNA constructs (shPFKB#09 and shPFKB#20) (Fig. 2d and Extended Data Fig. 3f) abolished pSRC-3-Ser857 levels in breast cancer cells cultured in 25 mM glucose, indicating that PFKFB4-dependent SRC-3 phosphorylation on Ser857 is a highly selective modification under conditions conducive to active glycolysis. We expressed the phosphorylation-defective mutant SRC-3(Ser857Ala) or wild-type SRC-3 protein in SRC-3-ablated cells, and under conditions of active glycolysis the levels of pSRC-3-Ser857 are increased in wild-type SRC-3 cells compared to the SRC-3(Ser857Ala) mutant cells (Extended Data Fig. 3g). Importantly, the introduction of fructose-1,6-bisphosphate (FBP) alone into glucose-starved cells permeabilized with streptolysin O rescued pSRC-3–Ser857 levels (Extended Data Fig. 3h), indicating that this phosphorylation event is linked to the energy status of the cell.

To measure the importance of this modification on the intrinsic activity of SRC-3, we transfected cancer cells with adenovirus expressing PFKFB4 (ad–PFKFB4) or control GFP, and cultured the transfected cells in the presence of normal glucose (25 mM) or low glucose (5 mM) levels. Enhanced expression of PFKFB4 along with glucose stimulation significantly increased the transcriptional activity of SRC-3 (pBIND–SRC-3) compared to cells cultured in low glucose conditions, suggesting that PFKFB4-dependent SRC-3 phosphorylation is important for the coactivator-driven transcriptional response (Extended Data Fig. 3i). To substantiate this observation, we used the phosphorylation-deficient pBIND–SRC-3(Ser857Ala) mutant or the phosphorylation-mimic pBIND–SRC-3(Ser857Glu) mutant in similar transcriptional activation assay and found that the Ser857Ala mutant was significantly refractory to glucose-dependent PFKFB4 signalling (Fig. 2e). The Ser857Glu mutant was constitutively active even at low levels of glucose, and glucose stimulation failed to show any further activation (Extended Data Fig. 4a). Previous studies have identified several crucial sites in the kinase domain of PFKFB4 that are important for ATP binding. When mutated to alanine, residues Gly46, Pro48, Gly51, Arg229 and Arg237 significantly decreased the binding affinity for ATP and result in reduced PFKFB4 kinase activity. We expressed these mutants in PFKFB4–silenced breast cancer cells and transcriptional assays confirmed significantly reduced SRC-3 activity and Ser857 phosphorylation (Extended Data Fig. 4b, c). Because SRC-3 is an established oestrogen receptor (ER) coactivator, we investigated the importance of glucose-dependent PFKFB4 signalling on ER-mediated transcriptional activity. MCF-7 cells stably expressing an oestrogen receptor (E2)–ER-dependent luciferase reporter gene (ERE–MAR–Luc) were used to assay ER activity as a function of E2 and glucose in the medium. Glucose addition enhanced ER activity, whereas low glucose or SRC-3 silencing significantly repressed transcriptional output in response to E2 (Extended Data Fig. 4d). Overexpression of PFKFB4 enhanced ER activity only in cells treated with E2 and glucose, whereas this PFKFB4-dependent increase in ER activity is repressed upon SRC-3 ablation (Extended Data Fig. 4e). Consistent with this observation, the SRC-3(Ser857Ala) mutant failed to rescue the growth of
SRC-3-depleted cells compared to wild-type SRC-3 (Extended Data Fig. 4f). These findings suggest that in glycolytic breast tumours, PFKFB4 and SRC-3 can also hyperactivate ER activity in the presence of E2, and phosphorylation of SRC-3 at Ser857 is a critical mark required for transcriptional responses.

PFKFB4 is an important regulator of glucose metabolism and directs metabolic pathways required for biosynthesis of macromolecules to sustain rapid proliferation in cancer cells. To identify the physiological role of PFKFB4-dependent SRC-3 activation in tumour metabolism, we performed an unbiased phenotypic screen to identify the metabolites that are preferentially used by SRC-3-overexpressing cells. For this we used a phenotype microarray analysis containing 93 metabolites (Supplementary Table 2) arrayed in a microplate and measured in real-time the importance of these metabolites in supporting SRC-3-dependent growth. We transduced mammary epithelial MCF10A cells (with relatively low endogenous SRC-3) with adenoviruses expressing GFP or SRC-3 followed by the phenotype screen for biologically independent experiments with similar results. See Source Data for exact P values.

To measure the direct contribution of PFKFB4 and SRC-3 regulation of glucose flux towards the pentose phosphate pathway (PPP), we used isotope-labelled [6-13C]glucose to trace the carbon flow. PFKFB4 and SRC-3 depletion significantly reduced the 13C-enrichment of ribulose-5P/xylulose-5P, important intermediary metabolites in the PPP and rate-limiting precursors for purine biosynthesis (Extended Data Fig. 6a). We investigated whether exogenous addition of purines could rescue the reduced growth rate of SRC-3-deficient breast cancer cells. As expected, loss of SRC-3 suppressed the growth of MDA-MB-231 and MCF-7 breast cancer cells, whereas supplementation of purines in the culture medium significantly rescued the growth defect, indicating that SRC-3 expression is crucial for the synthesis of purines for growth (Fig. 3b).

To identify the potential mechanisms of apparent SRC-3-driven purine synthesis, we performed gene expression analysis of enzymes involved in the PPP and purine synthesis. Knockdown of SRC-3 reduced the mRNA expression of transketolase (TKT), adenosine monophosphate deaminase 1 (AMPD1), and xanthine dehydrogenase (XDH) (Extended Data Fig. 6b, c). These SRC-3 target genes were also found to be regulated by PFKFB4 knockdown (Fig. 3c) and their expression was significantly enhanced in actively glycolytic breast cancer cells (Extended Data Fig. 6d). TKT is a major enzyme mediating non-oxidative PPP, whereas XDH and AMPD1 traditionally known to regulate purine catabolism are found to be regulated by SRC-3 (24, 25). Whether the switch in roles by these reversible enzymes XDH and AMPD1 depend on tumour metabolic state needs further investigation. Similarly, [6,13C]glucose isotope-tracing experiments also confirmed reduced levels of TKT products seduheptulose-7P and erythrose-4P upon PFKFB4 or SRC-3 knockdown (Extended Data Fig. 6e, f).

**Fig. 2 | PFKFB4 phosphorylates SRC-3 by functioning as a protein kinase.** a, Top, recombinant GST-fused PFKFB4 incubated with full-length SRC-3 in the presence of [32P]ATP in an in vitro kinase assay. Bottom, SRC-3 and PFKFB4 protein levels were analysed by immunoblotting. b, In vitro kinase assay of PFKFB4 in the presence of SRC-3 fragments expressing different domains or full-length (FL) SRC-3. c, HEK293T cells expressing Flag-tagged SRC-3 and PFKFB4 cultured in different concentrations of glucose and immunoprecipitated by Flag or pSer/Thr antibodies followed by immunoblotting. d, MDA-MB-231 cells stably expressing shRNAs targeting PFKFB4 (shPFK#09 and shPFK#20) or control non-targeting (NT) shRNA grown in the presence of 5 mM or 25 mM glucose, or after glucose withdrawal (WD), in which cells were cultured in 25 mM glucose for 24 h and then switched to 5 mM glucose for 6 h. Protein levels of pSRC-3-Ser857, PFKFB4 and β-actin were detected by immunoblotting. e, HEK293T cells expressing pBIND, pBIND-SRC-3 or pBIND-SRC-3(Ser857Ala) were transduced with adenoviruses expressing GFP or PFKFB4, and cultured in 5 mM or 25 mM glucose followed by luciferase assay. Boxes represent the twenty-fifth and seventy-fifth percentiles, lines represent median, whiskers showing minimum and maximum points, and plus symbol indicates the mean. n = 6 biologically independent experiments. ****P < 0.00001, two-way ANOVA with Tukey’s multiple comparisons test. Data in a–e are representative of three biologically independent experiments with similar results. See Source Data for exact P values.
To confirm that these genes are direct targets of SRC-3, we re-expressed SRC-3 in MDA-MB-231 cells with depleted levels of endogenous SRC-3 protein (Extended Data Fig. 3g) and observed significant restoration of SRC-3 target genes (Extended Data Fig. 7a). The addition of exogenous purines also restored the primary growth defects in PFKFB4-silenced MDA-MB-231 cells (Extended Data Fig. 7b), with a decreased incorporation of [U-13C]glucose carbon into purines (Extended Data Fig. 7c). Although the metabolic effects may or may not be directly regulated by target genes AMPD1 or XDH, our findings indicate that PFKFB4 and SRC-3 mutually cooperate to drive glucose flux towards purine generation.

To define how PFKFB4 phosphorylation of SRC-3 affects transcriptional regulation of the three commonly regulated purine biosynthesis genes defined above, we analysed the chromatin occupancy of SRC-3 on the promoters of TKT, XDH and AMPD1 using an existing in silico analysis of SRC-3 chromatin immunoprecipitation followed by sequencing (ChIP–seq) dataset26. We identified strong overlap of SRC-3 occupancy with activating transcription factor 4 (ATF4)-binding sites3 on the three target genes (Extended Data Fig. 8a, b). Interestingly, ATF4 has been recently identified to promote purine synthesis in response to growth signals28. To validate whether SRC-3 interacts with ATF4, we immunoprecipitated ATF4 from MDA-MB-231 cells growing in either 25 mM or 5 mM glucose. Under conditions of enhanced glycolysis, the interaction of ATF4 with pSRC-3-Ser857 increased robustly although the total ATF4 protein level was lower owing to reduced nutrient stress compared to 5 mM glucose treatment. However, the loss of PFKFB4, SRC-3, or re-expression of SRC-3(Ser857Ala) in SRC-3-knockdown cancer cells greatly reduced the association (Fig. 3d). Next we performed ChIP and quantitative PCR (ChIP–qPCR) to measure the chromatin occupancy of ATF4, pSRC-3-Ser857 and SRC-3 on the target gene promoters. Breast cancer cells growing in the presence of 25 mM glucose showed increased occupancy of ATF4 and pSRC-3-Ser857 on TKT (Fig. 3e), XDH (Extended Data Fig. 8c) and AMPD1 (Fig. 3f) promoters, whereas the loss of SRC-3 or PFKFB4 significantly reduced ATF4 chromatin occupancy on AMPD1 (Extended Data Fig. 8d). In addition, we found SRC-3 recruitment to the gene promoters is dependent on ATF4, as knockdown of ATF4 significantly reduced target gene expression and pSRC-3-Ser857 promoter occupancy (Extended Data Fig. 8e, f). These findings demonstrate that in actively glycolytic breast cancers, PFKFB4-dependent phosphorylation of SRC-3 at Ser857 promotes interaction with the transcription factor ATF4, thereby stabilizing the complex on chromatin and driving transcription of key metabolic enzymes.

To study whether suppression of PFKFB4 or SRC-3 can affect the growth of breast tumours in vivo (Fig. 4a), we implanted MDA-MB-231 cells stably expressing non-targeting shRNA, SRC-3 shRNA (Extended Data Fig. 3g) and PFKFB4 shRNA (Fig. 2d) into the mammary fat pad of female nude mice. Compared to control mice, genetic loss of SRC-3 or PFKFB4 exhibited substantially reduced tumour growth and volume (Fig. 4b and Extended Data Fig. 9a). Immunostaining with a human Ki67 antibody showed significantly reduced proliferative cells in SRC-3- or PFKFB4-ablated tumours compared to controls (Extended Data Fig. 9b, c). To evaluate the functional significance of the Ser857 phosphorylation of SRC-3 in breast tumour progression, we stably expressed shRNA-resistant wild type or SRC-3(Ser857Ala) in MDA-MB-231 cells with suppressed expression of endogenous SRC-3 protein (Extended Data Fig. 3g). Rescuing expression with the exogenous wild-type SRC-3 construct in SRC-3-depleted cells completely restored the growth of the breast tumours (Extended Data Fig. 9d), whereas the phosphorylation-deficient Ser857Ala mutant (Extended Data Fig. 9d, e) was partially resistant to tumorigenesis six weeks after grafting the tumour cells (Fig. 4b and Extended Data Fig. 9a).
After resecting out the primary tumours, we allowed the animals to survive for four more weeks with weekly bioluminescence imaging (Fig. 4a) to evaluate metastatic potential. Animals with primary tumours expressing wild-type SRC-3 developed profound lung metastasis with morbid hunched back posture, whereas suppression of SRC-3 or PFKFB4 or expression of the SRC-3(Ser857Ala) phosphorylation-deficient mutant all showed markedly reduced lung lesions (Fig. 4c and Extended Data Fig. 9f). Pathological analysis identified only a few micro-metastatic lesions in the lungs of animals with SRC-3(Ser857Ala), or SRC-3- and PFKFB4-ablated primary tumours (Fig. 4d), with no observed health issues during the four weeks after surgery. These findings demonstrate that SRC-3 and PFKFB4 are drivers of basal-subtype breast tumour growth and that phosphorylation of SRC-3 at the Ser857 site is crucial for metastatic progression of the disease. Immunostaining of the primary tumours with a pSRC-3-Ser857 antibody detected increased nuclear-localized human SRC-3 in the tumours collected from wild-type animals that progressed to aggressive metastatic disease, whereas PFKFB4- or SRC-3-ablated tumours had significantly reduced nuclear staining (Fig. 4e, f). Nuclear-localized pSRC-3-Ser857 represents active SRC-3 in the tumour that in turn promotes target gene expression to maintain tumour growth and metastasis. Importantly, this single phosphorylation site modification was also found to be an indicator of tumour metastasis mediated by ERK3 in a previous study. Taken together, our data demonstrate that the PFKFB4–SRC-3 signalling axis promotes tumour cell proliferation by increasing purine synthesis (Extended Data Fig. 9g), which may also serve as a critical determinant of metastatic progression of the disease.

To identify the clinical implications of this axis, we first analysed expression of PFKFB4 in The Cancer Genome Atlas (TCGA) database and found its expression to be significantly enhanced across all subtypes of breast cancer (Extended Data Fig. 9h). Because SRC-3 is an ER coactivator, we analysed expression of pSRC-3-Ser857 and PFKFB4 in ER-positive primary breast tumours and adjacent normal tissues. Our data show increased levels of pSRC-3-Ser857, PFKFB4 and SRC-3 in most tumours compared to normal tissues (Extended Data Fig. 10a, b), and a significant correlation between pSRC-3-Ser857 and PFKFB4 levels (r = 0.63, Extended Data Fig. 10c). Because PFKFB4 expression is also increased in other breast tumour subtypes, we performed protein array analyses using MDA-MB-231 cell lysates with suppressed expression of SRC-3 or PFKFB4 protein, and compared the significantly altered protein targets to the control non-targeting shRNA. Our study identified a common proteomic signature by intersecting the significant proteins affected by ‘both’ SRC-3 and PFKFB4 knockdown (Extended Data Fig. 10d). Imposing the restriction of protein–changes in the ‘same direction’ we evaluated the correlation of the common PFKFB4–SRC-3 proteomic signature with patient survival in a cohort of specimens from patients with breast cancer for which clinical information was available. We identified that the PFKFB4–SRC-3 common proteomic
signature also is associated with a decreased likelihood of survival in a basal-like-subtype triple-negative patient cohort (Extended Data Fig. 10e). These clinical associations are compatible with our in vivo experimental observations substantiating that the PFKFB4–SRC-3 axis is a molecular powerhouse that propels breast tumorigenesis leading it to an aggressive metastatic disease.

Here we have uncovered an interaction between the glycolytic pathway and the oncogenic activation of the transcriptional coactivator SRC-3. The Warburg effect is known to be one of the most dominant sugar metabolic pathways across cancers generating energy and macromolecules to sustain rapid proliferation and tumour growth. We now find that a glycolytic stimulator, the bifunctional enzyme PFKFB4, also can operate as a protein kinase, at least in actively glycolytic tumours.

After glucose uptake, PFKFB4 catalyses the synthesis of F2,6BP from F6P and ATP; and our study revealed that under these conditions, PFKFB4 can also phosphorylate SRC-3 at Ser857. Phosphorylation of SRC-3 at Ser857 rapidly increases its transcriptional activity and promotes the synthesis of genes for driving glucose flux towards purine synthesis (Extended Data Fig. 10f). The PFKFB4–SRC-3 axis was found to be enriched in ER-positive breast tumours, and was also identified to promote a common protemic signature that correlates with worse outcomes in patients with triple-negative breast cancer, thereby driving an aggressive metastatic disease (Extended Data Fig. 10g). Our work suggests that targeting the PFKFB4–SRC-3 axis may be therapeutically valuable in breast tumours that are notably dependent on glucose metabolism.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0018-1.

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Author contributions S.D. and B.W.O. conceived the project. S.D. performed most experiments with the following exceptions: N.P. independently performed metabolic profiling and isotope-tracing analysis, J.M.C. and S.Y.T. independently performed proteomics analysis and identified the phosphorylation site, and B.Z. analysed the cistromes and motif analysis. K.R. performed all the data analysis, statistical tests and human datasets for clinical correlation under the supervision of C.C. B.C.N. developed plasmids used in the study; C.E.F. validated the pSR3-3-Ser857 antibody, and P.X. purified full-length SRC-3 protein and recombinant SRC-3 fragments. T.F.W. provided the RNAi-knome library and supervised the library screen. X.H.-F.Z. supervised the animal study. B.W.O., M.-J.T. and S.Y.T. supervised the overall project. S.D. and B.W.O. conceived the project. S.D. performed...
METHODS

Vectors and virus production. Commercially-shRNA targeting the 3′ UTR region of the PFKFB4 (TRCN0000199909-sh9) and TRCN0000198920-sh20) and SRC-3 (TRCN0000370321-sh21 and TRCN0000365196-sh9) were obtained from Sigma. Lentiviruses were produced by transient transfection using Lipofectamine 2000 (Life Technologies) into 293 T cells along with pMD2.G (a gift from D. Trono, Addgene plasmid 12259) and psPAX2 (a gift from D. Trono, Addgene plasmid 12289) and the viral supernatants were collected after 48 h followed by precipitation and purification using PEG-IR Virus Concentration Solution (System Bioscience)10. The construct expressing the GAL4 active luciferase reporter (pG5-luc) was obtained from Promega, and the pBIND-SRC-3 construct was generated by inserting an in-frame fusion between the GAL4 DNA-binding domain and the open reading frame of human SRC-3, as previously described12. The pBIND-SRC-3 (SR357A)13 and pBIND-SRC-3 (SR357F) mutant were generated using the QuickChange Lightning site-directed mutagenesis kit, as described earlier13. The GST–SRC-3 fragment constructs were obtained by cloning portions of the SRC-3 (amino acids 1–320), serine/threonine (S/T) (amino acids 321–584), CID (amino acids 841–1080), and HAT (amino acids 1081–1421) domains generated by immunizing animals with a synthetic peptide containing the C-terminal HAT domain of SRC-3 by BioDesign. Stable cells expressing shRNAs were generated by lentiviral transduction in presenilin 1 (PS1)-expressing HeLa cells14. The shRNA sequences were as follows: shPFK-09 (TRCN0000199909): 5′-CCGGGTTGATTGCTGACACATTCTCCGTGAAAGAATGCGCGCAATCCA GCTTTTTTGG-3′; shPFK-20 (TRCN0000199920): 5′-CCGGGGCCGACGCTTTTA GGTGTTTCACCTCGAGGTGAACACCTAAGAGCTGCGCTTTTTTG-3′; shSRC-3 (TRCN0000370321): 5′-CCGGTTGCACCTGAAATGCGCGCAATCCA GCTTTTTTGG-3′; shPFK#09 (TRCN0000199909-sh09 and TRCN0000199920-sh20) were obtained from Origene (RC01573). The PFKFB4 mutants Gly46Ala, Pro48Ala, Gly51Ala, Arg236Ala and Arg238Ala were generated by site-directed mutagenesis. All constructs were verified by Sanger sequencing. The siGENOME siRNA against PFKFB4, SRC-3 and ATF4 were obtained from Dharmacon.

The shRNA sequences were as follows: shPFK-09 (TRCN0000199909): 5′-CCGGGTTGATTGCTGACACATTCTCCGTGAAAGAATGCGCGCAATCCA GCTTTTTTGG-3′; shPFK-20 (TRCN0000199920): 5′-CCGGGGCCGACGCTTTTA GGTGTTTCACCTCGAGGTGAACACCTAAGAGCTGCGCTTTTTTG-3′; shSRC-3 (TRCN0000370321): 5′-CCGGTTGCACCTGAAATGCGCGCAATCCA GCTTTTTTGG-3′; shPFK#09 (TRCN0000199909-sh09 and TRCN0000199920-sh20) were obtained from Origene (RC01573). The PFKFB4 mutants Gly46Ala, Pro48Ala, Gly51Ala, Arg236Ala and Arg238Ala were generated by site-directed mutagenesis. All constructs were verified by Sanger sequencing. The siGENOME siRNA against PFKFB4, SRC-3 and ATF4 were obtained from Dharmacon.

Cell culture. HeLa, HEK293T, MDA-MB-231, MCF-7 and MCF-7-ERE-MAR-Luc cells were cultured in DMEM (Gibco) supplemented with 10% FBS; SK-BR-3 cells were grown in McCoy’s medium with 10% FBS; and MCF-10A cells in DMEM/F12 (Gibco) supplemented with 5% horse serum, epidermal growth factor (EGF), hydrocortisone, cholera toxin and insulin. All cell lines were incubated at 37 °C and 5% CO2. Cell lines were obtained from ATCC, and maintained and yearly tested for mycoplasma contamination by the Tissue Culture Core, Baylor College of Medicine. Stable cells expressing shRNAs were generated by lentiviral transduction in presence of polybrene (8 µg/ml). Polyclonal pooled populations of stable cells were selected in the presence of puromycin (1 µg/ml) for more than three passages before initiating any functional experiments.

Human kinome library screen. A high-throughput RNAi screen was performed using the Stealth RNAi human kinase library (Life Technologies) targeting each of 636 human kinases with three individual siRNAs directed at different regions of the gene that were arrayed in twenty-four 96-well plates. To identify the kinases that modulate SRC-3 transcriptional activity, we re-veco-transfected HeLa cells with pBIND or pBIND-SRC-3 (2 ng per 96-well) along with pG5luc firefly-luciferase reporter (12,500 cells per 96-well) in complete growth medium (DMEM plus 10% FBS) on 96-well plates. After 48 h of culture, plates were carefully washed with PBS and luminescence (12,500 cells per 96-well) in complete growth medium (DMEM plus 10% FBS) on 96-well plates. After 48 h of culture, plates were carefully washed with PBS and luminescence was recorded in luminometer (Berthold) using the Dual-Luciferase Assay System (Promega). Additional well plates on all plates had appropriate controls containing cells transfected with pBIND and siRNA targeting GFP (sgGFP), or pBIND-SRC-3 and siGFP along with reporter plasmid. SRC-3 transcriptional activity was calculated by comparing the relative luciferase units (RLU) of pBIND-SRC-3 to pBIND readings transcribed with sgGFP. Firefly luciferase reading from each well was normalized to its Renilla reading (pBIND-vector backbone contains the Renilla luciferase gene) to adjust the variations in transfection efficiency. The fold change in SRC-3 activity upon suppression of kinases was calculated by comparing data to sgGFP readings, followed by robust z-score analysis to identify kinases that either increase or decrease SRC-3 activity more than 2 s.d. above or below. Differences in luciferase activity between pBIND-SRC-3 plus sgGFP and pBIND-SRC-3 plus siGFP values were converted to log2, for each set of siRNA and then graphed in 3D plot.

Cell proliferation assays. Cells were transfected with indicated siRNAs and were seeded at a density of 3,000 cells per 96-well in complete growth medium. For rescue experiment, cells were seeded in complete growth medium supplemented with dialysed serum with or without purines (10 µM adenosine, Sigma, and 10 µM guanosine, Sigma). After 4 days, cells were stained with CellTiter96 (Promega) reagent followed by measurement of absorbance at 490 nm. For the clonogenic survival assays, 1,000 cells per well were plated onto a 6-well plate, and were incubated for 7 days, and stained with crystal violet. The medium was changed every two days.

Cell culture treatment conditions, protein isolation and immunoblotting. For siRNA, cells were lysed 72 h after transfection. Stable cells were grown until 80% confluence before protein was extracted. During nutritional stress conditions, stable cells were cultured in complete medium until 80% confluence, followed by a brief starvation (3 h) in glucose-free growth medium. Cells were then switched to glucose-free DMEM supplemented with 10% dialysed serum and 5 mM or 25 mM glucose, as indicated in the figures, for 24 h before cells were lysed. For glucose withdrawal, cells were cultured in 25 mM for 24 h and then switched to medium containing 5 mM glucose for an additional 6 h. For FBP treatment, glucose-starved cells were pre-treated with 10 µM streptolysin O (Sigma, S5262) to permeabilize the cells, followed by the addition of FBP (Santa Cruz, sc-214805) as previously described13. Immunoblotting was performed as previously described14. In brief, cells were lysed using NP-40 lysis buffer (Life Technologies) along with protease and phosphatase inhibitor cocktail (Millipore). Total protein was estimated using a BCA protein estimation kit (Pierce) and approximately 40 µg protein was separated by 12% Bis-Tris gels (Life Technology) and electroblotted onto nitrocellulose membranes using the iBlot system (Life Technology). Blots were blocked for 2 h at room temperature or overnight at 4 °C in 1 × TBS buffer (Biorad) supplemented with 0.1% Tween-20 (Sigma) and either 5% bovine serum albumin (BSA) or 5% non-fat dry milk (Biorad). Blots were incubated overnight at 4 °C with primary antibody diluted into TBST containing 1% BSA or 5% non-fat dry milk. Blots were subsequently washed three times for 10 min in TBST and incubated with secondary antibody coupled to HRP (Promega). Blots were washed as previously described, reacted with ECL reagents (Thermo Fisher Scientific) and detected by chemi-luminescence (UVP Biospectrum). Semi-quantitative levels of each band were analysed by densitometry using UVP Vision Works LS software, and the relative values normalized to actin are indicated numerically under each lane.

Antibodies used for immunoblotting in the study are: mouse monoclonal SRC-3 (611105, BD Biosciences), rabbit monoclonal SRC-3 (2126, Cell Signaling), Flag (F3165, Sigma–Aldrich), mouse phosphoSerine/Threonine (612548, BD Biosciences), rabbit PFKFB4 (137785 and 71622, Abcam), mouse PFKFB4 (TA00809, Origene), rabbit monoclonal ATF4 (11815, Cell Signaling), and β-actin conjugated to HRP (A3854, Sigma–Aldrich). The phospho-SRC-3 (Ser857) antibody monoclonal antibody was cell culture supernatant produced from hybridoma generated by immunizing animals with a synthetic peptide containing phosphorylated Ser857 of human SRC-3. This antibody (Clone 10A6) was a gift from Cell Signaling Technology.

Immunoprecipitations. 293T cultured in 100 mm dishes until 80% confluence was transfected with Flag–SRC-3 following infection with adenovirus PFKFB4 (Signagen Laboratories). Twenty-four hours after infection, the medium was changed and cells were incubated overnight in different concentrations of glucose (5 mM or 25 mM) in glucose-free DMEM medium supplemented with 10% dialysed FBS. For MDA-MB-231 cells, stable cells expressing shRNAs targeting PFKFB4 or SRC-3 or expressing Ser857Ala in SRC-3 depleted cells were grown in 5 mM or 25 mM glucose. Cells were lysed in NP-40 lysis buffer (Innogenot).
supplemented with protease and phosphatase inhibitor cocktail (Millipore). For co-immunoprecipitations, lysates were precleared with control Protein A/G Agarose beads (Pierce). Five hundred micrograms of protein were then used for pull-down assays using monoclonal anti-Flag (F16156, Sigma) or anti-phospho-Ser/Thr antibody (BD Biosciences) overnight. The beads were then captured, washed and immunoprecipitated proteins were eluted and subjected to immunoblotting, along with 2% input sample run in parallel. For ATF4 pull-down, anti-ATF4 (11815, Cell Signalling) was used, the data were collected at ≥250 eV, respectively. Approximately 9–12 data points were acquired per detected metabolite.

Isotope labelling and profiling by targeted mass spectrometry. Glucose labelled with [6-13C]glucose and [U-13C]glucose were purchased from Cambridge Isotope Laboratories. MDA-MB231 cells were grown in 10-cm dishes in regular medium until 80% confluence, followed by brief (3h) starvation and then addition of 25mM of [6-13C]glucose supplemented with glucose-free DMEM medium with 10% dialysed FBS and 1% penicillin/streptomycin. For [U-13C]glucose, cells were fed with steady-state isotope tracers for 48h and medium was replaced 2h before metabolome collection and/or isotope tracer addition. Culture medium was collected, cells were washed with PBS, counted, and snap-frozen in liquid nitrogen. Cells were screened to 0.5 mM mixture of 1:1 water:methanol, sonicated for 1 min (two 30s pulses), and then mixed with 450 µl ice-cold chloroform. The resulting homogenate was then mixed with ice-cold water and vortexed again for 2 min. The homogenate was incubated at −20 °C for 30 min and centrifuged at 4 °C for 10 min to partition the aqueous and organic layers. The aqueous and organic layers were separated and dried at 37 °C for 45 min in an automatic Environmental Speed Vac system (Thermo Fisher Scientific). The extract was reconstituted in a 500 µl solvent containing-reduce methanol-water (1:1) and filtered through a 3-kDa molecular filter (Amicon Ultra-3 KDa Membrane) at 4 °C for 90 min to remove proteins. The filtrate was dried at 37 °C for 45 min in a speed vacuum and stored at −80 °C until mass spectrometry analysis. Before mass spectrometry analysis, the dried extract was reconstituted in 100 µl of ice-cold methanol-water (1:1) containing 0.1% formic acid and analysed using multiple reaction monitoring (MRM). Liquid chromatography–mass spectrometry HPLC analysis was performed using an Agilent 1290 series HPLC system equipped with a degasser, binary pump, thermostatted auto sampler and column oven (all from Agilent Technologies). The MRM-based measurement of relative metabolite levels were used for normal phase chromatographic separation. All samples were kept at 4 °C, and 5 µl of the sample was used for analysis.

Separation of TCA, glycolysis and PPP-associated metabolites. The normal phase chromatographic separation was also used for targeted identification of metabolites. This analysis used solvents containing water (solvent A), with a gradient spanning 80% B to 2% B over a 20-min period followed by 2% B to 80% B for a 5-min period and followed by 80% B for a 13-min time period. The flow rate was gradually increased during the separation from 0.2 ml min⁻¹ (0–20 min), 0.3 ml min⁻¹ (20–25 min), 0.35 ml min⁻¹ (25–30 min), 0.4 ml min⁻¹ (30–37.99 min) and finally set at 0.2 ml min⁻¹ (37.99 min). Metabolites were separated on a Luna Amino (NH2) column (4 µm, 100 A 2.1 × 150 mm, Phenomenex) that was maintained in a temperature-controlled chamber (37 °C). All the columns used in this study were washed and reconditioned after every 50 injections. Ten microlitres was injected and analysed using a 6495 QQQ triple quadrupole mass spectrometer (Agilent Technologies) coupled to a 1290 series HPLC system via selected reaction monitoring (SRM). Metabolites were measured using negative ionization mode with an electrospray ionization (ESI) voltage of −4000 eV, respectively. Approximately 9–12 data points were acquired per detected metabolite.

Isotope labelling and profiling by targeted mass spectrometry. Glucose labelled with [6-13C]glucose and [U-13C]glucose were purchased from Cambridge Isotope Laboratories. MDA-MB231 cells were grown in 10-cm dishes in regular medium until 80% confluence, followed by brief (3h) starvation and then addition of 25mM of [6-13C]glucose supplemented with glucose-free DMEM medium with 10% dialysed FBS and 1% penicillin/streptomycin. For [U-13C]glucose, cells were fed with steady-state isotope tracers for 48h and medium was replaced 2h before metabolome collection and/or isotope tracer addition. Culture medium was collected, cells were washed with PBS, counted, and snap-frozen in liquid nitrogen. Cells were screened to 0.5 mM mixture of 1:1 water:methanol, sonicated for 1 min (two 30s pulses), and then mixed with 450 µl ice-cold chloroform. The resulting homogenate was then mixed with ice-cold water and vortexed again for 2 min. The homogenate was incubated at −20 °C for 30 min and centrifuged at 4 °C for 10 min to partition the aqueous and organic layers. The aqueous and organic layers were combined and dried at 37 °C for 45 min in an automatic Environmental Speed Vac system (Thermo Fisher Scientific). The extract was reconstituted in a 500 µl solvent containing-reduce methanol-water (1:1) and filtered through a 3-kDa molecular filter (Amicon Ultra-3 KDa Membrane) at 4 °C for 90 min to remove proteins. The filtrate was dried at 37 °C for 45 min in a speed vacuum and stored at −80 °C until mass spectrometry analysis. Before MS analysis, the dried extract was resuspended in a 50 µl solution of methanol-water (1:1) containing 0.1% formic acid, and then analysed using MRM. Ten microlitres was injected and analysed using a 6490 QQQ triple quadrupole mass spectrometer (Agilent Technologies) coupled to a 1290 Series HPLC system via SRM. Metabolites were targeted in both positive and negative ion mode: the ESI voltage was 4,000 V in positive ion mode and −3,500 V in negative ion mode. Approximately 9–12 data points were acquired per detected metabolite. To target the TCA flux, the samples were delivered to the mass spectrometer via normal-phase chromatography using a Luna Amino column (4 µm, 100 A 2.1 × 150 mm). To target the fatty-acid flux, the samples were delivered to the mass spectrometer via reverse-phase chromatography using a Phenyl Hexyl column (3 µm, 100 A 2.1 × 150 mm). For 13C-labelled experiments, SRM was performed for expected 13C incorporation in various forms for targeted lipid chromatography–tandem mass spectrometry (LC–MS/MS). Mass isosporomer distribution (MID) was calculated and corrected for natural abundance. Proximity ligation assay.

Interaction between endogenous SRC-3 and PFKFB4 was detected using the PLA technique using Duolink In Situ Red Starter Kit Mouse/Rabbit (U09210, Sigma) according to manufacturer’s instructions. In brief, MDA-MB-231 cells were seeded in a 35-mm glass bottom culture dish (P35G-0-14C, MatTek Corporation), and after reaching 80% confluency, cells were fixed followed by blocking for 1h with the Duolink Blocking Solution at 37 °C. Cells were then incubated in presence of primary antibodies: SRC-3 (rabbit monoclonal, Cell Signaling) and PFKFB4 (mouse monoclonal, Origene), either alone or in combination. After incubation, cells were washed and Duolink PLA PLUS and MINUS probes were added for 1h at 37 °C. After washing off the unbound probes, cells were incubated first with the ligase enzyme followed by DNA polymerase enzyme to amplify the DNA circle. Finally, cells were mounted using Duolink In Situ Mounting Media with DAPI, and analysed by microscopy. Images were obtained using Zeiss Axios Observer A1 inverted microscope with N-Achromat 100 × /1.25 oil lens, Zeiss MRC5 camera, and AxioVision Rel.4.8 software.

Analysis of ATF4 and SRC-3 cistromes and motif analysis of ATF4-bound sequences. Owing to the lack of ATF4 and SRC-3 ChIP-seq datasets in breast cancer cell lines, we compared an in-house SRC-3 ChIP–seq dataset of mouse liver29, with previously published ATF4 ChIP–seq data in mouse embryonic fibroblasts27. Even though this comparison is less than ideal as SRC-3 and ATF4 ChIP–seq were performed in different tissues, the co-localization of SRC-3 and ATF4 cistromes even in different tissues, nevertheless, argues for an interplay between them, a finding subsequently confirmed by co-immunoprecipitation and ChIP-qPCR assay in human breast cancer cell lines. ATF4 binding motifs in the promoter regions of XDH, TKT and AMPD1 genes were discovered using the MISP (Motif-based Interval Screener with PSSM) toolbox in Galaxy Cistrome with a P value cut-off of 0.005. The consensus ATF4-binding motif used as input is TGTAGCACA. A similar strategy was used to investigate that ChIP–seq was in combination with the Agilent 2100 (Agilent Technologies) or Bioanalyzer 2100 (Agilent Technologies).
grown in 15-cm dishes until 80% confluent. For glucose stimulation, cells were glucose-deprived for 3 h by incubating in glucose-free DMEM supplemented with 10% FBS, followed by 4 h stimulation with 5 mM or 25 mM glucose. Cells were cross-linked in 1% formaldehyde and quenched with 125 mM glycine. Chromatin was sheared by sonication using a Branson Sonifier, precleared with control IgG antibodies and agarose beads (Millipore), and then immunoprecipitated with IgG (control), SRC-3, pSRC-3-S857 and ATF4 antibodies. DNA fragments were eluted from beads followed by reverse-crosslinking and purified DNA was used in qPCR reactions using SYBR green (Applied Biosystems) to determine the promoter occupancy. Melt curve analysis was performed to verify all SYBR green reactions produced a single PCR product.

**Luciferase assays.** Luciferase assays were performed from whole-cell lysates made in Cell Culture Lysis reagent (Promega) using the Luciferase Reporter Assay (Promega) and a Berthold 96-well plate reader. Luciferase values were normalized to the total protein level.

**Metabolomic phenotyping microarrays.** Screening was performed using 96-well plate phenotype microarrays (Biolog) containing 88 different carbon substrates and 5 nucleotides as the energy source33. MCF10A cells were infected with adenovirus expressing GFP or SRC-3, and seeded at an initial density of 2 × 10^4 cells per well in triplicate. Biolog Redox Dye Mix MA was added to each well according to the manufacturer's instructions, and kinetic usage of the metabolites was monitored using the GEN III OmniLog ID System (Biolog).

**Human breast tumours.** The breast tumours and adjoining normal tissue was obtained from the Lester and Sue Smith Breast Center at Baylor College of Medicine according to the Institutional Research Board approved protocol #H-7900. Whole-cell lysates from a total of 14 human breast tumours that are ER+ primary tumours, along with matched normal tissues, were used to detect pSRC-3-Ser857, SRC-3, and PFKFB4 levels by immunoblotting.

**Determining a common PFKFB4–SRC-3 proteomic signature.** Protein lysates from MDA-MB-231 cells stably expressing shRNAs targeting SRC-3 or PFKFB4 were used for protein array analysis as described before24. Expression of proteins significantly altered owing to the ablation of PFKFB4 and SRC-3 compared with non-targeting control shRNA were determined using a parametric t-test as implemented in the python (spicy) statistical system. Significance was assessed for P < 0.05, fold change exceeding 1.25 ×, and normalized signal levels exceeding 200 U. A common proteomic signature was determined by intersecting the significant proteins affected by each treatment, and imposing the restriction that the protein changes are in the same direction.

**Association of the PFKFB4–SRC-3 proteomic signature in human basal breast cancer.** We evaluated the association of the common PFKFB4–SRC-3 proteomic signature with patient survival in a cohort of primary basal breast cancer patient specimens collected by The Cancer Genome Atlas (TCGA) for which clinical information has been collected25. We first subsetted the proteins measured using the array by TCGA. Next, for each protein in the PFKFB4–SRC-3 common proteomic signature and for each basal breast cancer specimen, we computed the z-score for its expression within the patient cohort. We then computed the sum of the z-scores for each specimen. Specifically, the z-scores of the proteins suppressed by PFKFB4–SRC-3 (that is, upregulated by PFKFB4 and SRC-3 shRNA) were subtracted from the z-scores of the products induced by PFKFB4 (that is, downregulated by PFKFB4 and SRC-3 shRNA); this resulted in an activity score of the PFKFB4–SRC-3 common proteomic signature, respectively, for each specimen. After computing the activity scores, we further partitioned the patient cohort into specimens with a high activity score (top 33% of the specimens) and specimens with a low activity score (bottom 33% of the specimens) for the corresponding signatures.

We considered significant association with survival using the log-rank test (P < 0.05) and the Cox proportional hazard test (P < 0.05) available via the package survival as implemented in the R statistical system.

**Tumorigenicity and metastasis assays.** All animal experiments were carried out in accordance with a protocol approved by the Baylor College of Medicine Institutional Animal Care and Use Committee and experiments were terminated once maximal tumour volumes were reached (10% of the animal body weight). MDA-MB-231 breast cancer cells stably expressing luciferase were individually transduced with shRNAs targeting SRC-3 and PFKFB4. For the rescue experiment, SRC-3-ablated tumour cells were used to restore the levels of either wild-type SRC-3 or the SRC-3(Ser857Ala) mutant, and the polyclonal pooled population was selected. Approximately 2.5 × 10^6 cells were injected at orthotopic site along with Matrigel (BD Biosciences) (1:1 volume) in the mammary fat pad of 5–6 week-old female athymic nude Foxn1-nu mice (Envigo). The mammary tumour length (L) and width (W) were measured with a caliper. Tumour volumes were calculated using the formula V = L^2 × W/2. Six weeks after tumour resection animals were euthanized and tissues were collected and fixed in 4% PFA. Paraffin-embedded lungs samples were also subjected to haematoxylin and eosin staining to reveal the size and number of lung macro or micro-metastases. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment. No statistical methods were used to predetermine sample size estimate.

**Statistics.** Unless otherwise indicated, all results represent the mean ± s.d., and statistical comparisons between different groups were performed using the two-tailed Student’s t-test, one-way or two-way ANOVA with appropriate multiple comparisons corrections. For all statistical analyses, differences of P < 0.05 were considered statistically significant, and three biologically independent experiments with similar results are reported. GraphPad Prism software version 6.0/7.0 (GraphPad Software) was used for data analysis.

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

**Data availability.** The ChIP–seq data have been submitted to the Gene Expression Omnibus under accessions GSE35681 (for ATF4) and GSE67860 (for SRC-3). Other data that support the findings of this study are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Kinome-wide screen identified potential kinases regulating SRC-3 intrinsic transcriptional activity. a, HeLa cells expressing varying concentrations of the pBIND or pBIND-SRC-3 constructs were used to measure SRC-3 activity. n = 4 biologically independent samples. *P < 0.000001, one-way ANOVA with Sidak’s multiple comparison test. RLU are normalized by protein content. b, HeLa cells expressing pBIND or pBIND-SRC-3 were treated with siRNA targeting GFP or PRKCZ at the indicated dose followed by luciferase assay to measure SRC-3 activity. n = 3 biologically independent samples. *P < 0.000001, one-way ANOVA with Tukey’s multiple comparison test. c, Different control siRNAs targeting GFP or luciferase (Luc) were used to measure SRC-3 activity in HeLa cells expressing pBIND or pBIND-SRC-3. n = 3 biologically independent samples. The GFP control siRNAs in the red box were used in the library screen as controls. d, Effect on SRC-3 transcriptional activity by three sets of siRNA (sets A, B and C) targeting 636 human kinases in HeLa cells. Effect of GFP control siRNA was set at 1 (dotted line), the cut-off fold for increased activation was set at 2, and reduced activity at 0.75 following z-score analysis. n = 3 siRNAs per / kinase, n = 6 siGFP per plate; total n = 1,908 (siRNAs targeting kinases) n = 144 (siGFP control) independent samples. e, SRC-3 activity in HeLa cells across 24 kinome-library plates in the presence of control siRNA targeting GFP. n = 6 biologically independent replicates for each plate. f, A secondary screen was performed in HeLa cells to confirm the primary screen hits using a pooled siRNA targeting the kinases followed by SRC-3 transcriptional activity. n = 3 biologically independent samples. Boxes are as in Fig. 2e. g, Relative proliferation of MDA-MB-231 cells 4 days after treatment with siRNAs targeting GFP (control), SRC-3 or the indicated kinases. n = 3 biological replicates. *P < 0.0001. two-way ANOVA with Dunnett’s multiple comparisons test. Unless stated otherwise, data are mean ± s.d.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | PFKFB4, the top hit from the kinase screen, enhances the transcriptional activity of SRC-3. a, Effect of PFKFB4 knockdown on SRC-3 transcriptional activity in various breast cancer cell lines. $n = 3$ or $n = 4$ (siGFP plus pBIND-SRC-3) biologically independent cells. *$P < 0.000009$, two-way ANOVA with Tukey’s multiple comparison test. b, SRC-3 transcriptional activity in MDA-MB-231 cells expressing shRNAs targeting PFKFB4 (#09 and #20) or non-targeting control, co-transfected with pBIND or pBIND-SRC-3. $n = 5$, biological replicates. *$P < 0.0001$, one-way ANOVA with Tukey’s multiple comparisons test. c, Protein expression of PFKFB4 or actin in MDA-MB-231 cells expressing shRNAs targeting PFKFB4. d, Expression of PFKFB4 and SRC-3 mRNA in indicated breast tumour cells after treatment with siRNAs targeting GFP control or PFKFB4. $n = 4$ or $n = 3$ biological replicates. See Source Data for exact $P$ values. e, Expression of PFKFB4 and SRC-3 mRNA in MDA-MB-231 cells transduced with adenoviruses expressing GFP or PFKFB4. $n = 6$ biologically independent cells. ***$P < 0.000001$, two-way ANOVA with Tukey’s multiple comparison test. f, Left, MDA-MB-231 cells were stained with specific antibodies against SRC-3 (rabbit) and PFKFB4 (mouse) before proximity ligation assay (PLA). The PLA signals between endogenous SRC-3 and PFKFB4 are shown in the red channel, DAPI was used to stain the nuclei (blue) and the merge images show the overlay of the red and blue channels. Two representative fields from biologically independent experiments were shown from $n = 5$. Right, control cells were stained with either one of the antibodies against SRC-3, PFKFB4 or secondary antibody-conjugated with probes. Scale bars, 20 µm (left), 40 µm (right). Data are representative of three biologically independent experiments with similar results, and are shown as mean ± s.d.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | PFKFB4 functions as a protein kinase by phosphorylating SRC-3 at the Ser857 residue. a, In vitro PFKFB4 kinase assay in the presence of purified SRC-3 protein, F6P, ATP and increasing concentration of recombinant PFKFB4 enzyme followed by SDS–PAGE. Immunoblotting with pSer/Thr antibody shows the level of phosphorylated SRC-3 protein. b, In vitro PFKFB4 kinase assay in presence of purified SRC-3 protein, PFKFB4 enzyme and varying concentrations of F6P and ATP followed by SDS–PAGE. Immunoblotting with pSer/Thr antibody shows the level of pSRC-3 protein. c, Coomassie blue stain showing the levels of GST-fused SRC-3 fragments used in in vitro kinase reactions performed in Fig. 2b. d, Proteomics analysis of in vitro kinase assay using the GST–SRC-3-CID fragment in the presence of PFKFB4 enzyme and ATP followed by mass spectrometric analyses. Mass spectrum shows the green phosphorylation peak. e, Proteomics analysis of an in vitro kinase assay using a Ser857Ala-mutated GST–SRC-3-CID protein in the presence of PFKFB4 enzyme and ATP, followed by mass spectrometric analyses. Mass spectrum failed to detect phosphorylation peaks in the Ser857Ala-mutated SRC-3-CID protein. f, Expression of PFKFB1, PFKFB2, PFKFB3 and PFKFB4 in MDA-MB-231 cells expressing shRNAs targeting PFKFB4 (#09 and #20). mRNA levels were normalized to internal housekeeping gene ACTB. n = 3 biological replicates. *P < 0.05, two-way ANOVA with Tukey’s multiple comparisons test. g, Protein levels of pSRC-3-Ser857, total-SRC-3 and actin in MDA-MB-231 cells stably expressing non-targeting control shRNA, SRC-3 shRNA, or SRC-3 shRNA plus the shRNA-resistant Ser857Ala SRC-3 mutant (shSRC-3 + S857A) or SRC-3 shRNA plus wild-type SRC-3 (shSRC-3 + WT-SRC-3) cultured in 25 mM glucose. Protein bands were quantified by ImageJ after normalization to β-actin. h, MDA-MB-231 cells stably expressing non-targeting shRNA or shRNA targeting PFKFB4 were grown in the presence of 25 mM glucose or were glucose-starved for 4 h followed by incubation with streptolysin O for 5 min. FBP (10 µM) was added to glucose-starved cells for an additional 1 h, followed by cell lysis and immunoblotting. Protein bands were quantified by ImageJ after normalization to β-actin and the non-targeting shRNA lane was set to 1. i, Relative luciferase activity showing the transcriptional activity of SRC-3 in MDA-MB-231 cells transduced with adenoviruses expressing GFP or PFKFB4 cultured in the presence of 5 mM, 15 mM or 25 mM glucose. n = 6 (pBIND) and n = 3 (pBIND-SRC-3) biological cell samples. *P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. Data in a–c, f–h are representative of three biologically independent experiments with similar results, and in d, e are representative of two biologically independent experiments each run with three different reactions all showing similar results and peptide coverage. Data are mean ± s.d.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Ser857 phosphorylation enhances SRC-3 transcriptional activity. a, Relative luciferase activity showing the activity of wild-type SRC-3, and the Ser857Ala and Ser857Glu SRC-3 mutants in MDA-MB-231 cells transduced with lentivirus expressing non-targeting shRNA or PFKFB4 shRNA cultured in the presence of 5 mM or 25 mM glucose. n = 3 biological cell samples. *P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. b, Relative luciferase activity showing the activity of SRC-3 in MDA-MB-231 cells stably expressing lentivirus PFKFB4 shRNA and cultured in the presence of 25 mM glucose. The cells are then co-transfected with empty vector, wild-type PFKFB4 and PFKFB4 mutants Gly46Ala, Pro48Ala, Gly51Ala, Arg230Ala and Arg238Ala. n = 6 biological cell samples. *P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. c, MDA-MB-231 cells stably expressing shRNAs targeting PFKFB4 (231-shPFKFB4) were transfected with constructs expressing empty vector (vector), wild-type PFKFB4, and PFKFB4 mutants Gly46Ala, Pro48Ala, Gly51Ala, Arg230Ala and Arg238Ala, and cultured in presence of 25 mM glucose. Protein levels of pSRC-3-Ser857, PFKFB4 and β-actin were detected by immunoblotting. d, Relative luciferase activity showing the activity of oestrogen receptor-α (ERα) in MCF7-Mar-luc cells transduced with lentivirus expressing non-targeting shRNA or SRC-3 shRNA cultured in the presence of 5 mM or 25 mM glucose stimulated with 100 nm E2, or with ethanol control (−E2). n = 3 biological cell samples. *P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. e, Relative luciferase activity showing the activity of ERα in MCF7-Mar-luc cells transduced with adenovirus expressing GFP or PFKFB4. Cells transduced with PFKFB4 adenovirus were infected with non-targeting shRNA or SRC-3 shRNA after 2 days and then cultured in the presence of 5 mM or 25 mM glucose stimulated with ethanol (−E2) or with 100 nM E2. n = 3 biological cell samples. *P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. f, Survival assay in MCF7 cells showing the effect of non-targeting shRNA, SRC-3 shRNA, and re-expression of wild-type SRC-3 or SRC-3(Ser857Ala) mutant in SRC-3-depleted cells cultured in charcoal-stripped medium supplemented with 25 mM glucose and E2 for 7 days. n = 3 biological independent data are shown. All data are representative of three independent experiments with similar results, and shown as mean ± s.d.
Increased glucose and purines are required for SRC-3-dependent growth. a, Real-time measurement of MCF10A cell proliferation transduced with adenoviruses expressing GFP or SRC-3 in the presence of 93 different metabolites. $n = 3$ independent plates run for each sample. b, Relative growth of MCF10A cells transduced with adenoviruses expressing GFP or SRC-3 in the presence of $\alpha$-glucose (b), adenosine (c) and inosine (d). $n = 6$ biological cell samples. **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, unpaired t-test two tailed. Boxes are as in Fig. 2e. e, f, Relative levels of intermediary metabolites in MDA-MB-231 cells after treatment with shRNAs targeting PFKFB4 or SRC-3 compared to control shRNA. e, Glycolytic and PPP metabolites. f, Nucleotides. $n = 3$ biological independent samples. *$P < 0.05$, two-way ANOVA with Tukey’s multiple comparisons test. g, Total levels of purines in MCF10A cells transduced with with adenoviruses expressing GFP or SRC-3. $n = 3$ biological independent samples. *$P < 0.05$, ***$P < 0.001$, two-way ANOVA with Tukey’s multiple comparisons test. See Source Data for exact $P$ values. Unless stated otherwise, data are mean ± s.d.
Extended Data Fig. 6 | SRC-3 drives the purine synthesis program under conditions of active glycolysis. a, MDA-MB231 cells stably expressing control shRNA, PFKFB4 shRNA and SRC-3 shRNA were fed with [6-13C]glucose. Ribulose/xylulose-5P (m + 1) labelling from [6-13C]glucose is shown. n = 3 biological cell samples. ***P = 0.00013, ****P = 0.000078, one-way ANOVA with Tukey’s multiple comparisons test. b, Genes involved in oxidative and non-oxidative PPP. n = 3 biological cell samples. *P = 0.043, two-way ANOVA with Sidak’s multiple comparisons test. c, Genes involved in nucleotide synthesis. n = 3 biological cell samples. *P < 0.05, **P < 0.01, ***P < 0.001, two-way ANOVA with Sidak’s multiple comparisons test. d, mRNA expression of the metabolic enzymes TKT, XDH and AMPD1 in MDA-MB-231 cells transduced with adenovirus expressing GFP (control) and PFKFB4 cultured in the presence of 5 mM, 15 mM or 25 mM glucose. n = 3 biological cell samples. **P < 0.01, ****P < 0.001, two-way ANOVA with Dunnett’s multiple comparisons test. e, f, MDA-MB231 cells stably expressing control shRNA, PFKFB4 shRNA and SRC-3 shRNA were fed with [6-13C]glucose. Seduheptulose-7P (m + 1) (e) and erythrose-4P (f) labelling from [6-13C]glucose are shown. n = 3 biological cell samples. **P < 0.01, ****P = 0.001, two-way ANOVA with Dunnett’s multiple comparisons test (e) or with Tukey’s multiple comparison test (f). Boxes are as in Fig. 2e. See Source Data for exact P values. Unless stated otherwise, data are mean ± s.d.
Extended Data Fig. 7 | Growth defect due to loss of SRC-3 or PFKFB4 is rescued by exogenous purines. a, Expression of the metabolic enzymes encoded by TKT, XDH, AMPD1 and SRC-3 in MDA-MB-231 cells expressing control shRNA, SRC-3 shRNA or SRC-3 shRNA plus re-expression of shRNA-resistant wild-type SRC-3 protein (shSRC-3-21+WT-SRC-3). n = 4 biological cell samples. *P < 0.05, **P < 0.01, ***P < 0.001, ****P = 0.0001, two-way ANOVA with Tukey’s multiple comparisons test.
b, Relative proliferation of MDA-MB-231 cells expressing shRNA targeting SRC-3 (shSRC-3#01 and shSRC-3#02) or non-targeting control shRNA after treatment with siRNAs targeting luciferase (siLuc; as a control) or PFKFB4 under the conditions indicated. n = 6 samples from biologically independent experiments. ****P < 0.000001, two-way ANOVA with Tukey’s multiple comparisons test. c, MDA-MB231 cells stably expressing control shRNA, PFKFB4 shRNA or SRC-3 shRNA were fed with [U-13C]glucose for 48 h. Adenosine 13C-labelling from [U-13C]glucose is shown. n = 3 samples from biologically independent experiments. one-way ANOVA with Tukey’s multiple comparisons test. Boxes are as in Fig. 2e. Data are representative of three biologically independent experiments with similar results. See Source Data for exact P values. Unless stated otherwise, data are mean ± s.d.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | PFKFB4–SRC-3 stabilizes ATF4 transcription factor to promote purine synthesis. a, Chromatin localization peaks of SRC-3 and ATF4 on Tkt, Xdh and Ampd1 genes in mouse liver. b, ATF4-binding peaks are conserved on three SRC-3 target purine biosynthetic genes in both mouse and human genomes. c, Chromatin immunoprecipitation (ChIP) of ATF4, total SRC-3 and pSRC-3-Ser857 from MDA-MB-231 cells treated with 5 mM or 25 mM glucose compared to an IgG isotype control. qPCR was performed to determine amount of promoter enrichment. d, ChIP–qPCR was performed from MDA-MB-231 cells cultured in 25 mM glucose expressing SRC-3 shRNA, PFKFB4 shRNA or control shRNA. n = 3 biological cell samples. *P < 0.01, **P < 0.001, ***P < 0.0005, ****P < 0.00001, one-way ANOVA with Tukey’s multiple comparisons test compared to 5 mM glucose groups (c) and compared to NT shRNA group (d). e, ChIP of ATF4, total SRC-3 (BD Biosciences antibody), and pSRC-3-Ser857 from MDA-MB-231 cells on the AMPD1 promoter treated with non-targeting siRNA or siRNA against ATF4, and cultured in presence of 25 mM glucose compared to an IgG isotype control. qPCR was performed to determine the amount of promoter enrichment. n = 3 biological cell samples. ***P < 0.001, ****P < 0.00001, one-way ANOVA with Tukey’s multiple comparisons test. f, mRNA expression of TKT, XDH, AMPD1 and SRC-3 in MDA-MB-231 cells expressing siRNA targeting control or ATF4 siRNA. n = 3 biological cell samples. two-way ANOVA with Sidak’s multiple comparisons test. See Source Data for exact P values. Data are mean ± s.d.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | The PFKFB4–SRC-3 axis promotes breast tumour growth and metastasis. a, Primary tumours resected out after 6 weeks. b, Ki67 staining of primary tumours from animals injected with MDA-MB-231 cells stably expressing control shRNA, SRC-3 shRNA or PFKFB4 shRNA. Data are representative of five fields per slide from \( n = 5 \) animals per group with similar findings. Scale bar, 100 µm. c, Quantification of Ki67-positive cells in the tumour. \( n = 5 \) animals per group, average of five fields counted from each slide. ****P = 0.0001, one-way ANOVA with Dunnett’s multiple comparisons test. d, Primary tumour growth in animals injected with MDA-MB-231 cells stably expressing shRNA targeting SRC-3, PFKFB4, or expression of wild-type SRC-3 or the Ser857Ala mutant in the SRC-3-depleted cells. \( n = 5 \) animals per group. *P < 0.00001, two-way ANOVA with Tukey’s multiple comparisons test. e, Immunoblot showing the relative expression of SRC-3 in primary tumours from MDA-MB-231 cells stably expressing control shRNA, SRC-3 shRNA, or after re-expression of wild-type SRC-3 or the Ser857Ala mutant in the SRC-3-depleted cells. \( n = 5 \) animals per group was pooled to generate the tumour lysate used for analysis. f, Graph representing the photon flux of animals from different groups. \( n = 5 \) animals for wild-type SRC-3, the Ser857Ala mutant and PFKFB4 shRNA, and \( n = 4 \) animals for SRC-3 shRNA. *P < 0.0001, one-way ANOVA with Dunnett’s multiple comparisons test. Line shows median with range. g, mRNA expression of three metabolic enzymes (TKT, XDH and AMPD1), SRC-3 and PFKFB4 from the primary tumours. \( n = 5 \) animals per group. **P < 0.05, ***P < 0.001, ****P = 0.0001, two-way ANOVA with Tukey’s multiple comparisons test. h, Expression of PFKFB4 in patients with breast cancer across different subtypes compared to normal breast tissue. Normal basal = 17; basal = 139; normal_Her2 = 9; Her2 = 67; normal luminal A = 62; luminal A = 418; normal luminal B = 21 and LumB = 186. Line in the centre of the rectangle represents the median, top edge of the rectangle represents the third quartile, bottom edge of the rectangle represents the first quartile, top whisker represents the maximum and bottom whisker represents the minimum. All data are representative of three biologically independent experiments with similar results, and are shown as mean ± s.d. unless otherwise stated. See Source Data for exact P values.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | The PFKFB4–SRC-3 axis drives transcriptional programming in patients with breast cancer. a, b, Expression of pSRC-3, SRC-3 and PFKFB4 in ER+ breast tumour specimens and matched adjoining normal tissues as detected by immunoblotting. n = 14 patients with ER+ breast cancer. c, Semi-quantitative levels of bands shown in a and b, analysed by densitometry using UVP Vision Works LS software, and normalized relative to actin to calculate the fold change (tumour/normal) and plotted to obtain the correlation between PFKFB4 and pSRC-3-Ser857 expression. n = 14 normal and tumour tissues. R = 0.63, P = 0.02 Spearman’s rank correlation coefficient. d, log fold change in protein expression of the PFKFB4–SRC-3 signature compared to the control knockdown (non-targeting shRNA) as determined using a parametric t-test as implemented in the python (spicy) statistical system. Significance P < 0.05 and fold change exceeding 1.25 × were used to classify true regulators of SRC-3 activity. n = 3 biologically independent samples. e, Kaplan–Meier survival plot showing poor survival of patients with breast cancer with basal subtype (triple-negative) disease exhibiting an increased expression of a common proteomic signature induced by the PFKFB4 and SRC-3 axis. The cohort of patients was collected by the TCGA. P = 0.0365, log-rank test; P = 0.02971, Cox proportional hazards, two-sided. f, Cartoon model describing the crosstalk between glycolysis and purine generation highlighting the essential steps regulated by pSRC-3-Ser857. This PFKFB4-dependent SRC-3 phosphorylation enhances mRNA expression of genes involved in purine metabolism driving breast tumour growth, proliferation and metastasis. AICAR, 5-aminimidazole-4-carboxamide ribonucleotide; AMP, adenosine monophosphate; F1,6-P, fructose 1,6 biphosphate; IMP, inosine monophosphate. g, Model showing that, in glycolytic breast tumours, activated PFKFB4 drives SRC-3 phosphorylation at Ser857, which then activates ER-positive primary tumour growth in conjunction with E2-liganded ER, as well in ER-negative/recurrent tumours in conjunction with ATF4, driving aggressive metastatic disease. Data are mean ± s.d.
## Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - Statistical methods were not used to predetermine sample size (n). Number of sample was determined based on experimental approach, availability, feasibility required to obtain definitive results.

2. **Data exclusions**
   - Describe any data exclusions.
   - None.

3. **Replication**
   - Describe the measures taken to verify the reproducibility of the experimental findings.
   - Experiments reported are replicated at least twice with similar observations.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - Majority of the experiments were performed following genetic manipulation of cells using shRNA/siRNA procedures or transfection of DNA plasmids. Similarly, animal experiments were performed with genetically manipulated cell lines. This design does not need randomization and recording the origin of samples are critical.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - The researchers were not blinded during data collection or analysis.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |
   | ☒   | ☒         |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
  - Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

*See the web collection on statistics for biologists for further resources and guidance.*
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

| Software                        |
|---------------------------------|
| Graphpad Prism 7                |
| AxioVision Rel.4.8              |
| R 3.4.2                         |
| Python 2.7                      |
| MISP (Motif-based Interval Screener with PSSM) version 0.1.0 |
| Image J Version 1.51t           |

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

| Materials availability |
|------------------------|
| No unique materials used. |

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

| Antibodies                                      |
|------------------------------------------------|
| Mouse monoclonal SRC-3 (Cat# 611105, BD Biosciences) PMID: 9252329 |
| Rabbit monoclonal SRC-3 (Cat# 2126, Cell Signaling) PMID: 17574025 |
| Mouse monoclonal SRC-3:5857 Clone 10A6 (Validated by Cell Signaling, kind gift) |
| Mouse FLAG (Cat# F3165, Sigma-Aldrich) PMID 18723513 |
| Mouse phosphoSerine/Threonine (Cat# 612548, BD Biosciences) PMCID: PMC3377643 |
| Rabbit PFKFB4 (Cat# 137785 and 71622, Abcam) PMID: 25115398 |
| Mouse PFKFB4 (Cat# TA500809, Orgene-Validated by manufacturer) |
| Rabbit monoclonal ATF4 (Cat# 11815, Cell Signaling)PMID: 29230015 |
| Mouse β-actin conjugated to HRP (Cat# A3854, Sigma-Aldrich) PMID 20679398 |
| Ki-67 antibody MIB-1 (Dako, #M7240) PMID: 29084952 |

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

| Eukaryotic cell lines |
|-----------------------|
| All lines were obtained from ATCC and maintained by Baylor Cell Culture Core Facility. |

b. Describe the method of cell line authentication used.

| Method of cell line authentication used |
|----------------------------------------|
| Cell lines obtained from ATCC are already authenticated using STR profiling. Lines that are obtained from other sources are maintained by Baylor Cell Culture Core Facility and undergo authentication by Short Tandem Repeat (STR) method at the M.D. Anderson ‘Characterized Cell Line Core Facility. All cell lines mentioned above undergo annual authentication by comparing the STR profile against a database of known STR profiles. M.D. Anderson Core Facility has STR profiles from many different commercial sources, and they compare against the known commercial list. Last authentication was done in 2017. |

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell lines are maintained under the recommended culture conditions and media requirements. Mycoplasma detection is performed by the Baylor Cell culture CORE every 3 months using the MycoAlert kit from Lonza. The last test was done on Oct 6th, 2017 and the test results for all the cell lines were negative.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

None.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

5-6 week-old female athymic nude Foxn1-nu mice (Envigo).
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

| Number | AgeGroup | ERGroup | PRGroup | Interp | HER2Group |
|--------|----------|---------|---------|--------|-----------|
| 1      | >=50 yrs | >2(+)   | >2(+)   | Equivocal(Allred2To6; CAP=2) |
| 2      | >=50 yrs | >2(+)   | <=2(-)  | Equivocal(Allred2To6; CAP=2) |
| 3      | >=50 yrs | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 4      | >=50 yrs | >2(+)   | >2(+)   | Equivocal(Allred2To6; CAP=2) |
| 5      | >=50 yrs | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 6      | >=50 yrs | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 7      | >=50 yrs | >2(+)   | >2(+)   | Equivocal(Allred2To6; CAP=2) |
| 8      | >=50 yrs | >2(+)   | >2(+)   | Equivocal(Allred2To6; CAP=2) |
| 9      | >=50 yrs | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 10     | >=50 yrs | >2(+)   | >2(+)   | Positive(AllredIn7,8; CAP=3) |
| 11     | >=50 yrs | >2(+)   | >2(+)   | Positive(AllredIn7,8; CAP=3) |
| 12     | <50 yrs  | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 13     | >=50 yrs | >2(+)   | >2(+)   | Negative(Allred=0; CAPin0,1) |
| 14     | >=50 yrs | >2(+)   | >2(+)   | Positive(AllredIn7,8; CAP=3) |