Supplementary Materials and Methods

Reagents and chemicals. Bovine serum albumin (BSA), Hank's balanced salt solution (HBSS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, dextran, sodium pyruvate, puromycin, basic-FGF, crystal violet, Triton™ X-100, Tween™ 80, (Hydroxypropyl)methyl cellulose, chloroquine diphosphate salt, calcium chloride dehydrate, polybrene were from Sigma-Aldrich (Buchs, Switzerland). Tissue-Tek OCT Sakura was from Sysmex Digitana AG (Horgen, Switzerland). Para-formaldehyde (PFA) was from Electron Microscopy Science (Hatfield, PA, USA). Dulbecco's Modified Eagle Medium (DMEM) GlutaMAX™, phosphate-buffered saline (PBS), simethylsulfoxice (DMSO), non-essential amino acids (NEAA), Fetal Bovine Serum (FBS), penicillin-streptomycin (P/S), L-glutamine, trypsin, were purchased from Gibco, Life Technologies - Invitrogen (Basel, Switzerland). Nilotinib (Receptor Tyrosine Kinase inhibitor) HC hydrate was purchased from MedKoo Biosciences (Chapel Hill, NC, USA). Collagenase type I was from Worthington Biochemical Corporation (Lakewood, NJ, USA) and DNase I from Roche (Indianapolis, IN, USA). D-luciferin firefly (potassium-salt) was purchased from Biosynth (Staad, Switzerland) or from FluoProbes, Interchim (Montluçon, France).

Cells and cell culture. The 4T1 parental murine mammary adenocarcinoma cell line was kindly provided by Prof. Dr. Fred R. Miller (Michigan Cancer Foundation, Detroit, MI (USA)) in 2008. 4T1 cells were cultured in high glucose DMEM supplemented with 10% heat-inactivated FBS, 1% P/S and 1% NEAA in a humidified incubator at 37 °C (5% CO_2, 20% O_2).

The D2A1 murine mammary adenocarcinoma cell line was kindly provided by Prof. Dr. Jonathan Sleeman (Medical Faculty Mannheim of the University of Heidelberg, Germany). D2A1 cells were cultured in high glucose DMEM supplemented with 10% heat-inactivated FBS and 1% P/S in a humidified incubator at 37 °C (5% CO_2, 20% O_2).

The MDA-MB-231 parental and the MDA-MB-231-BrM_2, brain metastatic, human breast cancer cell lines were kindly provided by Prof. Dr. Joan Massagué (Memorial Sloan Kettering Cancer Center, New York, USA) in (2016) as previously described (1). MDA-MB-231 cells were cultured in high glucose DMEM supplemented with 10% heat-inactivated FBS, 1% P/S in a humidified incubator at 37 °C (5% CO_2, 20% O_2).

The HEK 293T (ATCC, human embryonic kidney cells containing the SV-40 T-antigen) cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% P/S and were used for the production of recombinant lentiviral particles. They were cultivated in a humidified incubator at 37 °C (5% CO_2, 20% O_2).

Cell lines are tested for Mycoplasma contamination by PCR on NaOH cell lysates using the following Forward (ACTCCTACGGGAGGCAGCAGTA) and Reverse (TGCACCATCTGTCACTCTGTTAACCTC) primers at 58°C annealing temperature. Last testing : December 2019. Cell lines were not independently authenticated. Cell lines were typically for 3-4 months in the experiments before new stock aliquots were thawed.

Cell viability/death assay. Cell death and viability detection was performed using an APC-Annexin V Apoptosis Detection Kit (559763, BD Bioscience, Allschwil, Switzerland) according to manufacturer's instructions. For determination of living cells, the gate was set on $Annexin^-/PI^-$ cells. Data were acquired using MACSQuant

Analyzer (Miltenyi Biotec Swiss AG, Solothurn, Switzerland) and data analyzed by FlowJo v10.0.7 software.

Cell proliferation assay. Cells were collected, resuspended at 10^6 cells/ml in complete medium, incubated with 10 µM of Green CFSE (Life Technologies – Invitrogen, Basel, Switzerland) for 30 minutes at 37°C (5% CO_2, 20% O_2), washed to remove the excess of dye and then returned to culture. Dye fluorescence intensity was detected by flow cytometry at culture start (time 0), and at 24 and 48 hours later. Proliferation index is defined as the total number of divisions divided by the number of cells that underwent division and was calculated using FlowJo's software proliferation platform.

Cell growth assay. Cells were collected and seeded in tissue culture 96-well-plates (Costar, Milian AG, Wohlen, Switzerland) at 1,000 cells/well. Cells were allowed to grow in complete medium for 0, 24, and 48 hours in a humidified incubator at 37 °C (5% CO_2, 20% O_2) in presence of chemical reagents at the indicated concentrations. Cells were washed once with PBS prior to fixation with 4% PFA. Cells were stained with 0.5% crystal violet solution for 25 minutes at room temperature before excess dye was washed away. Absorbance was read at 595 nm for optical density (OD) calculation.

Cell hypoxia survival assay. Cells were collected and seeded in tissue culture 96-well-plates (Costar, Milian AG, Wohlen, Switzerland) at 10'000 cells/well. Cells were allowed to grow in complete medium for 0, 24, and 48 hours in a humidified incubator at 37 °C (5% CO_2, 0.1% O_2). Cells were washed once with PBS prior to fixation with

4% PFA and stained with 0.5% crystal violet solution for 25 minutes at room temperature before excess dye was washed away. Absorbance was read at 595 nm for optical density (OD) calculation.

Microvessel density (MVD) count. Tumors and brains were harvested at the end of the experiment, fixed in formalin and embedded in paraffin or fixed in OCT. 5 µm thick serial sections were cut from the tissue blocks. 3-4 sections at 100 µm distance per tumor were stained with CD31 antibody (Abcam ab28364). Stained slides were scanned by Nanozoomer (Hamamatsu Photonics) and analyzed by NDP.viewer2 software (Hamamatsu Photonics) for MVD using Chalkley method (2). Briefly, a grid with 25 randomly positioned dots was applied to 20 regions of scanned tumors (200X magnification). Dots on $CD31^+$ cells were counted as positive.

Quantitative real-time PCR. The mRNA expression levels were determined by semi-quantitative real-time polymerase chain reaction (RT-PCR). RNA samples were obtained from adherent cells using RNeasy kit from QIAGEN (Basel, Switzerland) according to manufacturer's instructions. From each sample, 1 µg RNA was retro-transcribed using SuperScript II Reverse Transcriptase kit (Life Technologies – Invitrogen (Basel, Switzerland), according to manufacturer's instructions. Real-time qPCR was performed using specific primers listed below (Microsynth AG, Balgach, Switzerland). The PCR reaction was performed in a StepOnePlus™ thermocycler (Applied Biosystems. Life Technologies, Basel, Switzerland) using the KapaSYBR® FAST SYBR Green Master Mix (Kapa Biosystems, Wilmington, USA). Each reaction was performed in triplicate and values were normalized to murine 36β4 or the human 18s ribosomal RNA housekeeping genes. The comparative Ct method was used to

calculate the difference of gene expression between samples as previously described (3).

TaqMan real-time PCR. Total RNA from subsets was extracted with the miRNAVana kit (Ambion of Applied Biosystems, Foster City, CA, USA), and mature microRNAs (miRNA-155 and controls RNU44 and snoRNA202) were reverse transcribed with TaqMan RT MicroRNA Kit (Applied Biosystems) and amplified by using Universal Fast Start Rox Probe Master Mix (Roche) and microRNA assay kits in 384-well plates (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 7900 HT device (Applied Biosystems, Foster City, CA, USA).

Real-time PCR primers

Gene	Access. N°	Forward primer	Reverse primer
(m) PDGFB	NM_011057	TATGAAATGCTGAGC GAACCA	CCTCGAGATGAGCTT TCCAA
(h) PDGFB	NM_002608	GGCGCTCTTCCTGTC TCTCTGCT	GTCCGAATG GTCACC CGAGTTTG
(m) PDGFRA	NM_011058	AGAAAATCCGATACC CGGAG	AGAGGAGGAGCTTGA GGGAG
(m) PDGFRB	NM_008809	TGGTATCACTCCTGG AAGCC	AACAGAAGACAGCGA GGTGG
(m) HIF-1α	NM_010431	GGCGAGAACGAGAAG AAAAA	GAGCTCACATTGTGG GGAAG

(m) CA9	NM_139305	GTGCCTATGAGCAGT TGCTGTC	AAGTAGCGGCTGAAG TCAGAGG
(h) CA9	NM_001216	GTGCCTATGAGCAGT TGCTGTC	AAGTAGCGGCTGAAG TCAGAGG
(m) VEGFA	NM_001025 250.3	TTACTGCTGTACCTCC ACC	ACAGGACGGCTTGAA GAT
(h) VEGFA	NM_001171 626	CACACAGGATGGCTT GAAGA	AGGGCAGAATCATCA CGAAG
(m) GLUT1	NM_011400	GAGTGTGGTGGATGG GATG	AACACTGGTGTCATC AACGC
(h) GLUT1	NM_006516	TTGCAGGCTTCTCCA ACTGGAC	CAGAACCAGGAGCAC AGTGAAG
(m) 36β4	NM_007475	GTGTGTCTGCAGATC GGGTAC	CAGATGGATCAGCCA GGAAG
(h) PDGFA	NM_002607	CAGCGACTCCTGGAG ATAGACT	CGATGCTTCTCTTCCT CCGAATG
(m) PDGFA	NM_008808	CCTCACCTGGACCTC TTTCA	TAACACCAGCAGCGT CAAGT
(h) PDGFRA	NM_006206	GCTCAGCCCTGTGAG AAGAC	ATTGCGGAATAACAT CGGAG
(h) PDGFRB	NM_002609	CAGGAGAGACAGCAA CAGCA	AACTGTGCCCACACC AGAAG
(h) HIF-1α	NM_001243 084	TGGCTGCATCTCGAG ACTTT	GAAGACATCGCGGGG AC

(h) 18s rib. RNA	NR_003286.2	CGACGACCCATTCGAACGTCT	GCTATTGGAGCTGGAATTACCG
(m/h) hsa-miR-let7d	MIMAT0021699	AGAGGUAGUAGGUUGCAUAGU	
(m/h) hsa-miR-let7f	MIMAT0000067	UGAGGUAGUAGAUUGUAUAGUU	
(m) sno-RNA202	AF_357327	GCTGTACTGACTTGATGAAAGTACTTTTGAACCCTTTTCCATCTGATG	
(h) RNU44	NR_002750	CCTGGATGATGATAGCAAATGCTGACTGAACATGAAGGTCTTAATTAGCTCTAACTGACT	

Lentiviral constructs. The small hairpin RNA to silence Pdgfb (shPdgfb) construct was purchased together with a non-silencing control sequence (shNS) from Sigma-Aldrich (Buchs, Switzerland). They were cloned into the pLKO.1-puro lentiviral vector. The (m) HIF-1α and the (h) HIF-1α shRNA sequences were kindly provided by PD Dr. Lubor Borsig (University of Zürich, Switzerland).

Target Gene	Species	shRNA N°	shRNA sequence
shNS (ctrl)	mouse/human	-	Scrambled sequence
shPDGFB	mouse	N°32	CGGCTGCTGCAATAACCGCAA
shHIF-1α	mouse	N°1	CCCATTCCTCATCCGTCAAAT
shHIF-1α	mouse	N°2	GCCACTTTGAATCAAAGAAAT
shHIF-1α	human	N°1	CGGCGAAGTAAAGAATCTGAA
shHIF-1α	human	N°2	CCGCTGGAGACACAATCATAT

The micro-RNA sequences were obtained from Biosettia (San Diego, CA, USA). The constructs were cloned into a pLV-[micro-RNA] expression vector.

The firefly luciferase plasmid was kindly provided by Prof. Dr. Michel Aguet, ISREC, Lausanne, Switzerland.

MicroRNA	Species	Accession N°	shRNA sequence
miR-000 (ctrl.)	mouse/human	-	scrambled sequence
miR-let-7d-5p	mouse/human	MI0000405	AGAGGUAGUAGGUUGCAUAGUU

Production of recombinant lentiviral and retroviral particles. In order to obtain recombinant viral particles, expression vectors and packaging vectors (Env, Pol, Gag) were co-precipitated with $CaCl_2$ as previously described (3). The precipitate was gently added to HEK-293T cells cultured at 30-50% confluence. Upon overnight transfection, the medium was replaced with fresh complete medium. Viral particle containing supernatant was collected after 24 and 48 hours and passed through a 0.45 µm syringe filter before it was directly used for transduction.

Transduction of cells with lentiviral constructs. In order to silence gene expression, cells were transduced with shRNA-expressing recombinant lentiviral particles for 24 hours in presence of polybrene (8 µg/ml). 48 hours post-transduction, stably transduced cells were selected with puromycin (5 µg/ml, 4 day selection). To stably overexpress micro-RNA let-7d, cells were transduced with recombinant lentiviral particles in presence of polybrene (8 µg/ml) and were selected with puromycin (5 µg/ml) as previously described (3).

Luciferase reporters. The Cignal™ luciferase Lenti Reporters to monitor the transcriptional activity of the transcription factor HIF-1α in mammalian cell lines were purchased from SABiosciences, QIAGEN (Basel, Switzerland). A positive control (CLS-PCL) and a negative control virus (CLS-NCL) were purchased from the same company. Cells were prepared according to the manufacturer's instructions. Briefly, tumor cells were grown in wells of a 96-well-plate to 30-50% confluence. For the transduction, 50 µl of lentiviral supernatant were added to each well and cells were incubated for 12 hours. In order to select for stably transduced cells, complete cell culture medium containing 5 µg/ml puromycin was added for 4 days. *In vitro* bioluminescence imaging was performed to assess the amount of luciferase expressed by cells in response to transcription factor activity.

Protein Isolation and western blot analysis. Cell lysis of cultured cells was performed in complete RIPA buffer containing phosphatase inhibitors (phenylmethylsulfonylfluoride (PMSF), sodium orthovanadate (Na_3VO4) and a phosphatase-inhibitor PI-Mix from Sigma-Aldrich (Buchs, Switzerland) and phosphatase substrate (beta-gycerophosphate disodium salt hydrate (BGP)). The protein lysates were subjected to careful sonication to shear cellular and molecular aggregates. A Bradford assay (Bio-Rad, Hercules, CA, USA) was used according to the manufacturer's instructions to determine the total protein concentration. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions with a 10% (proteins >30 kDa) or a 15% (proteins <30kDa) poly-acryl amide gel. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Merck Millipore, Darmstadt, Germany), blocked with 5% BSA (1

h at room temperature) and incubated overnight at 4°C with primary antibodies. Immunoreactivity on the membrane was visualized on photo-sensitive films using the relevant HRP-coupled secondary antibodies (1 h at room temperature) and the detection reagent (Luminata Classico Western HRP substrate, Merck Millipore, Darmstadt, Germany).

Antibodies

Anti-	Specificity	Host	Clonality	Cat. N°	Company
HIF-1α	mouse, human	rabbit	pAb	NB100-479	Novus Bio.
PDGFB	mouse, human	rabbit	pAb	Ab23914	Abcam
PDGFRB	mouse, human	rabbit	mAb	28E1	Cell Signaling
CD31	Mouse human	rabbit	pAb	Ab28364	Abcam

Bioluminescent imaging. BLI was performed using the IVIS Lumina II (PerkinElmer Life Sciences, Zürich, Switzerland). Firefly luciferase was stably transduced by lentiviral infection (LV-Luc) into parental tumor cells before selection. For *in vitro* BLI, luciferase-expressing tumor cells (LV-Luc) were collected and seeded in complete medium in black 96-well-plates (Nunc, Milian AG, Wohlen, Switzerland) as indicated and allowed to adhere. Prior to imaging, 300 µg/ml firefly D-luciferin substrate was

added to the culture medium. To measure the luciferase activity of transcription-factor reporters, cells were lysed with Passive Lysis Buffer (5X) from Promega (Fitchburg, USA). The lysate was transferred into wells of a black 96-well-plate (Nunc, Milian AG, Wohlen, Switzerland). Beetle-Juice luciferase reaction buffer (containing D-luciferin and ATP) from PJK (Kleinblittersdorf, Germany) was added before luciferase activity was measured. For *in vivo* BLI, mice received an intra-peritoneal injection of 150 mg/kg D-luciferin firefly in PBS 15 minutes prior to imaging. During the imaging procedure mice were anesthetized by isoflurane inhalation. For *ex vivo* BLI, mice received an intra-peritoneal injection of 150 mg/kg D-luciferin firefly in PBS 15 minutes prior to sacrifice. Organs were resected and placed in a 3 cm dish containing 300 µg/ml D-luciferin firefly in PBS and were incubated 10 minutes in dark prior to imaging. Images were acquired, analyzed and quantified using the Living Image Software (PerkinElmer Life Sciences, Zürich, Switzerland) by defining a region-of-interest (ROI) where the emitted Total Flux (photon/sec) was measured.

References to Supplementary Materials and Methods

1. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX*, et al.* Genes that mediate breast cancer metastasis to the brain. Nature **2009**;459:1005-9
2. Fox SB, Leek RD, Smith K, Hollyer J, Greenall M, Harris AL. Tumor angiogenesis in node-negative breast carcinomas--relationship with epidermal growth factor receptor, estrogen receptor, and survival. Breast Cancer Res Treat **1994**;29:109-16
3. Lan Q, Peyvandi S, Duffey N, Huang YT, Barras D, Held W*, et al.* Type I interferon/IRF7 axis instigates chemotherapy-induced immunological dormancy in breast cancer. Oncogene **2019**;38:2814-29