>19 corresponding to sites localized with >99% certainty. From four biological replicates, the application of stringent criteria selected 22 top-scoring candidate substrates of F3–T3 that exhibited at least a 1.5-fold enrichment in F3–T3 human astrocytes compared to human astrocytes expressing F3–T3(K508M) or the empty vector (Supplementary Table 2).

Identification of PIN4 complexes by mass spectrometry. Endogenous cellular PIN4 complexes were purified from the cell line H1299 transduced with the F3–T3-expressing lentivirus. Cellular lysates were prepared in 50 mM Tris-HCl, 250 mM NaCl, 0.2% NP40, 1 mM EDTA, 10% glycerol, protease and phosphatase inhibitors. PIN4 and mock immunoprecipitates were recovered with PIN4 antibody (Abcam, ab155283) and rabbit IgG, respectively. Immunocomplexes captured on protein A/G agarose beads were washed with lysis buffer containing 300 mM NaCl and 0.3% NP40. Bound polypeptides were eluted with the PIN4 peptide used as the epitope for the PIN4 antibody (KPVFTDPPVKTKFGYH, Abcam, ab155283). Eluates were run on SDS–PAGE gels and four gel slices were cut from the lane containing PIN4 immunoprecipitates (columns A1, B1, C1, D1; Supplementary Table 8). Four similar gel slices were cut from the lane containing control rabbit IgG immunoprecipitates (columns A2, B2, C2, D2; Supplementary Table 8). The excised gel pieces were rehydrated and digested in 80 μl of 12.5 ng μl^{-1} Trypsin Gold and 50 mM ammonium bicarbonate at 37 °C overnight. Extracted peptides were dried, reconstituted in 30 μl 0.1% TFA and stored at −20 °C before analysis.

The concentrated peptide mix was reconstituted in a solution of 2% acetonitrile (ACN), 2% formic acid (FA) and eluted from the column using a Dionex Ultimate 3000 Nano LC system. The application of a 2.0-kV distal voltage electrosprayed the eluting peptides directly into the Thermo Fusion Tribrid mass spectrometer equipped with an EASY-Spray source (Thermo Scientific). Mass spectrometer-scanning functions and HPLC gradients were controlled by the Xcalibur data system (Thermo Finnigan). Tandem mass spectra from raw files were searched against a human protein database using the Proteome Discoverer 1.4 software (Thermo Finnigan). The peptide mass search tolerance was set to 10 p.p.m. A minimum sequence length of seven amino acids residues was required. Only fully tryptic peptides were considered. Spectral counts were used for estimation of relative protein abundance between samples analysed directly on long gradient reverse phase liquid chromatography–tandem mass spectrometry. A specificity score of proteins interacting with PIN4 was computed for each polypeptide as described[50]. In brief, we compared the number of peptides identified from our mass spectrometry analysis to those reported in the CRAPome database that includes a list of potential contaminants from affinity purification–mass spectrometry experiments (http://www.crapome.org/). The specificity score is computed as $(p \times c) / S_{av} \times S_{max} \times E$, where p is the identified peptide count; c the cross-correlation score for all candidate peptides queried from the database; S_{av} the averaged spectral counts from CRAPome; S_{max} the maximal spectral counts from CRAPome; and E the total number of experiments that were found in the CRAPome database.

Cell culture. *Human cell lines.* h-TERT-immortalized human astrocytes[51], SF126 cells[52], U87 (ATCC HTB-14), h-TERT-RPE-1 (ATCC CRL-4000), HEK293T (ATCC CRL-11268), U251 (Sigma 09063001) cells. Cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS, Sigma). Cells were transfected using Lipofectamine 2000 (Invitrogen) or the calcium phosphate method.

Mouse glioma stem cells. F3–T3;shTrp53 and HRAS(12V);shTrp53 mGSCs were isolated from the brains of mice that had received injection of lentivirus containing a bi-cistronic expression cassette including F3–T3 or HRAS(12V) and *Trp53* shRNA into the dentate gyrus as described[4,7]. Mice showing neurological symptoms were euthanized 2–4 months after intracranial injection, and brain tumours were identified macroscopically, dissected and cultured in DMEM:F12 containing 1× N2 and B27 supplements (Invitrogen) and human recombinant FGF2 and EGF (20 ng ml^{-1} each; Peprotech). Studies were approved by the IACUC at Columbia University (AAAL7600).

Human glioma stem cells. The GBM-derived glioma stem cells (GSCs) used in this study have been described previously[4,12]. GBM-derived GSCs were grown in DMEM:F12 containing 1× N2 and B27 supplements (Invitrogen) and human recombinant FGF2 and EGF (20 ng ml^{-1} each; Peprotech). Cells were transduced using lentiviral particles in medium containing 4 μg ml^{-1} of polybrene (Sigma). Cells were routinely tested for mycoplasma contamination using the Mycoplasma Plus PCR Primer Set (Agilent Technologies) and were found to be negative. Cell authentication was performed using short-tandem repeats (STR) at the ATCC facility.

Limiting dilution assay (LDA) for human GSCs was performed as described previously[50]. In brief, spheres were dissociated into single cells and plated into 96-well plates in 0.2 ml of medium containing growth factors at increasing densities (1–100 cells per well) in triplicate. Cultures were left undisturbed for 14 days, and then the percentage of wells not containing spheres for each cell dilution was calculated and plotted against the number of cells per well. Linear regression lines were plotted, and we estimated the minimal frequency of glioma cells endowed with stem cell capacity (the number of cells required to generate at least one sphere in every well = the stem cell frequency) based on the Poisson distribution and the intersection at the 37% level using Prism 6.0 software. Data represent the means of three independent experiments performed on different days.

The soft agar colony assay was performed by seeding human astrocytes at a density of 10,000 cells per well in 6-well plates in 0.3% agar in DMEM and 10% FBS. The number of colonies per well was determined using an Olympus 1X70 microscope equipped with a digital camera.

Subcutaneous xenograft glioma models. Mice were housed in a pathogen-free animal facility. All animal studies were approved by the IACUC at Columbia University (AAAQ2459; AAAL7600). Mice were 4–6 week old male and female athymic nude (*Nu/Nu*, Charles River Laboratories). No statistical method was used to pre-determine sample size. No method of randomization was used to allocate animals to experimental groups. Mice in the same cage were generally part of the same treatment. The investigators were not blinded during outcome assessment. In none of the experiments did tumours exceed the maximum volume allowed according to our IACUC protocol, specifically, 20 mm in the maximum diameter. 5×10^5 F3–T3 human astrocytes transduced with a lentivirus expressing the shRNA sequence against *PPARGC1A* or *ESRRG* or the empty vector were injected subcutaneously in the right flank in 150 μl of saline solution (five mice per group). 0.5×10^5 F3–T3;shTrp53 mGSCs and HRAS(12V);shTrp53 mGSCs transduced with lentivirus expressing two independent shRNA sequences against *PPARGC1A* were injected subcutaneously in the right flank in 100 μl of saline solution (five mice per group). Treatment with tigecycline (10 mice) or vehicle (8 mice) was performed in mice injected with 1×10^5 F3–T3;shTrp53 mGSCs when tumours reached 150–270 mm^3 (10 days after injection). Tigecycline was diluted in saline pH 7 and administered at dose of 50 mg kg^{-1} body weight by intraperitoneal injection b.i.d. Tumour diameters were measured daily with a caliper and tumour volumes estimated using the formula: (width^2 × length) / 2 = V (mm^3). Mice were euthanized when tumour size reached the maximum diameter allowed by our IACUC protocol (20 mm in the maximum diameter) or when mice displayed body weight loss equal to or greater than 20% of total body mass, or showed signs of compromised health or distress.

Plasmids, cloning, and lentivirus production. cDNAs for *FGFR3*, *PIN4*, *PKM2*, *GOLGIN84*, *DLG3*, *C1ORF50* and *PPARGC1A* were amplified by PCR and cloned into the pLOC vector in frame with Flag or V5 tag. *F3–T3*, *F3–T3*^{K508M} and FLAG-tagged *PEX1* were cloned into a pLVX-puro vector (Clontech). To generate PIN4(Y122A), PIN4(Y122F), PKM2(Y105A), GOLGIN84(Y42A), DLG3(Y673A) and C1orf50(Y131A), site-directed mutagenesis was performed using the QuickChange Site-Directed mutagenesis kit (Agilent) and the resulting plasmids were sequence verified. Lentivirus was produced by co-transfection of the lentiviral vectors with pCMV-ΔR8.1 and pCMV-MD2.G plasmids into HEK293T cells as previously described[7,16]. shRNA sequences are: *PIN4* shRNA: GTCAGACACATTCTATGTGAACTCGAGTTCACATAGAATGTGTCTGAC; *PPARGC1A* Hs-shRNA1: GCAGAGTATGACGATGGTATTCTCGAGAA TACCATCGTCATACTCTGC; *PPARGC1A* Hs-shRNA2: CCGTT ATACCTGTGATGCTTTCTCGAGAAAGCATCACAGGTATAACGG; *Ppargc1a* Mm-shRNA1: CCAGAACAAGAACAACGGTTTCTCGAGAAACCGT TGTTCTTGTTCTGG; *Ppargc1a* Mm-shRNA2: CCCATTTGAGAACAAGAC TATCTCGAGATAGTCTTGTTCTCAAATGGG; *ESRRG* shRNA1: CAAACAA AGATCGACACATTGCTCGAGCAATGTGTCGATCTTTGTTTG; *ESRRG* shRNA2: CATGAAGCGCTGCAGGATTATCTCGAGATAATCCTGC AGCGCTTCATG.

For the generation of *PPARGC1A*-knockout F3–T3 human astrocytes with CRISPR–Cas9, guide RNA (gRNA) sequences were designed to target the coding sequence of *PPARGC1A* as described (http://crispr.mit.edu/). We designed two gRNAs against exon 1 of the *PPARGC1A* gene and validated three clones for loss of PGC1α expression. sgRNA sequence 1 (GGCGTGGGACATGTGCAACC) and 2 (ACCAGGACTCTGAGTCTGTA) were inserted by linker cloning in the lentiviral vector pLCiG2[53,54]. Human astrocytes expressing the empty vector or F3–T3 were infected either with pLCiG2 and control, pLCiG2 and *PPARGC1A* gRNA1 or pLCiG2 and *PPARGC1A* gRNA2. After 72 h of infection, cells were seeded in a 96-well plate at a density of 0.6 cell per well. Two weeks later, colonies were isolated and *PPARGC1A* deletion was analysed by RT–qPCR and western blot. For acute expression of F3–T3 in human astrocytes, cells were first transduced with pLOC expressing Flag–PIN4(WT) or the Y122F mutant. Subsequently, cells were transduced with a pLKO.1-puro vector encoding shRNA targeting *PIN4*. The levels of endogenous and ectopically expressed proteins were then verified by immunoblotting. Finally, cells were transduced with a pLVX vector expressing F3–T3.

Generation of phospho-PIN4(Y122) antibody. The anti-phospho-PIN4 antibody was generated by immunizing rabbits with a short synthetic peptide