containing phosphorylated Y122 (underlined) (PVKTKFGYHIIMVE) (Yenzym Antibodies, LLC). A two-step purification process was applied. First, antiserum was cross-absorbed against the phospho-peptide matrix to purify antibodies that recognized the phosphorylated peptide. Next, the anti-serum was purified against the un-phosphorylated peptide matrix to remove non-specific antibodies. Antibodies were validated using lysates from cells transfected with PIN4(WT) or the phospho-mutant PIN4(Y122A).

Immunoblot and immunoprecipitation. For western blot, cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP40, 0.5% sodium dexoycholate, 0.1% sodium dodecyl sulphate, 1.5 mM Na_3VO_4, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 10 mM β-glycerolphosphate and EDTA-free protease inhibitor cocktail, Roche). Lysates were cleared by centrifugation at 15,000 r.p.m. for 15 min at 4 °C. Phospho-tyrosine immunoprecipitation was performed on cells that were freshly collected in cold PBS containing Na_3VO_4 and lysed in RIPA buffer. Subsequently, 800 μg of protein extract was incubated with 30 μl of phospho-tyrosine sepharose beads (P-Tyr-100, Cell Signaling Technology, 9419) in a final volume of 800 μl overnight at 4 °C. Beads were washed five times with cold RIPA buffer and eluted by 2× SDS sample buffer. Immunoprecipitates were separated by SDS–PAGE and transferred to a nitrocellulose membrane. Membranes were blocked in TBS with 5% non-fat milk and 0.1% Tween-20, and probed with primary antibodies overnight at 4 °C. PIN4 and Flag–PEX1 immunoprecipitation was performed on cells that were freshly collected in cold PBS and lysed in 50 mM Tris pH 8.0, 150 mM NACl, 0.5% NP40, 1 mM EDTA, 10% glycerol, protease and phosphatase inhibitors. Subsequently, 2,000 μg of protein extract was incubated with PIN4 antibody (Abcam, ab155283) at a concentration of 0.6 μg mg^{-1} cell lysate or Flag–M2 agarose beads in a final volume of 1,000 μl overnight at 4 °C. For PIN4 immunoprecipitation, Protein A/G Plus agarose beads (Santa Cruz Biotechnology) were added for 2 h at 4 °C. Beads were washed five times with cold lysis buffer including 300 mM NaCl, and immunocomplexes were eluted with PIN4 or Flag–M2 peptide at room temperature for 4 h or 45 min, respectively. Immunoprecipitates were separated by SDS–PAGE and transferred to a nitrocellulose membrane. Membranes were blocked in TBS with 5% non-fat milk and 0.1% Tween-20, and probed with primary antibodies overnight at 4 °C.

Antibodies and concentrations were: FGFR3 1:1,000 (Santa Cruz, B-9, sc-13121), PIN4 1:1,000 (Abcam, ab155283), PKM2 1:1,000 (Cell Signaling, 3198), DLG3 (also known as SAP102) 1:1,000 (Cell Signaling, 3733), GOLGIN84 1:2,000 (Santa Cruz, H-283, sc-134704), C1orf50 1:1,000 (Novus Biologicals, NBP1-81053), HGS 1:1,000 (Abcam, ab72053), FAK 1:1,000 (Cell Signaling, 3285), Paxillin 1:1,000 (BD Transduction, 610051), PGC1α 1:500 (Santa Cruz, H300, sc-13067), PGC1α 1:1,000 (Novus Biological, NBP104676), ERRγ 1:500 (Abcam, ab128930), ERRγ 1:500 (R7D, PP-H6812000), phospho-FRS2 1:1,000 (Cell Signaling, 3861), FRS2 1:1,000 (Santa Cruz, sc-8318), phospho-STAT3 1:1,000 (Cell Signaling, 9131), STAT3 1:1,000 (Santa Cruz, C-20, sc-482,), phospho-AKT 1:1,000 (Cell Signaling, 4060), AKT 1:1,000 (Cell Signaling, 9272), phospho-ERK1/2 1:1,000 (Cell Signaling, 4370), ERK1/2 1:1,000 (Cell Signaling, 9102), β-actin 1:2,000 (Sigma, A5441), PEX1 1:500 (BD Biosciences, 611719), PEX6 1:500 (Stress Marq, SMC-470), NUP214 1:500 (Abcam, ab70497), SEC16A 1:500 (Abcam, ab70722), DHX30 1:500 (Novus Biologicals, NBP1-26203), SUN-2 1:500 (Abcam, ab124916), Flag 1:1,000 (Abcam, ab1162), retinoblastoma 1:1,000 (BD Pharmingen, 554136), α-tubulin 1:2,000 (Sigma, T5168), total OXPHOS 1:1,000 (Abcam, ab110411), MTCO1 1:1,000 (Abcam, ab14705). Secondary horseradish-peroxidase-conjugated antibodies were purchased from Pierce and Enhanced ChemiLuminescence (Amersham) or Super Signal West Femto (Thermo Scientific) was used for detection.

RT–qPCR. Total RNA was prepared using the Trizol reagent (Invitrogen) and cDNA was synthesized using SuperScript II Reverse Transcriptase (Invitrogen) as described[16,55]. RT–qPCR was performed with a Roche 480 thermal cycler, using SYBR Green PCR Master Mix (Applied Biosystems). RT–qPCR results were analysed by the $\Delta\Delta C_t$ method[56] using *18S* or *Actb* as the housekeeping gene.

Human primers used for RT–qPCR were as follows. UQCRC1 forward 5′-CACCGTGATGATGCTCTACC-3′ and reverse 5′-CCACCACCATAAGTGCAGTC-3′; *POLRMT* forward 5′-TATTCATGGTGAAGGATGCC-3′ and reverse 5′-TCTGTTCCAGACACCTTTCG-3′; *NDUFB4* forward 5′-TGCTTCAGTACAACGATCCC-3′ and reverse 5′-CACACAGAGCTCCCATGAGT-3′; *MRPL15* forward 5′-TGCTTCCACCAGAAGAACTG-3′ and reverse 5′-ACTTCCTGGCGAGTTCAAGT-3′; *MCL1* forward 5′-GCATCGAACCATTAGCAGAA-3′ and reverse 5′-TGCCACCTTCTAGGTCCTCT-3′; *MRPS30* forward 5′-TATTCCTCGTGGTCATCGAA-3′ and reverse 5′-CTCTGCGAGTTGCTTGGATA-3′; *TIMM10* forward 5′-CCTGGACCGATGTGTCTCTA-3′ and reverse 5′-GCACCCTCTTCATCAGCTCT-3′; *NRF1* forward 5′-GGAAACGGCCTCATGTATTT-3′ and reverse 5′-TCATCTAACGTGGCTCGAAG-3′; *ATP5G3* forward 5′-CCCAGAATGGTGTGTCTCAG-3′ and reverse 5′-TTCCAATACCAGCACCAGAA-3′; *ABCE1* forward 5′-TCATTGATCAAGAGGTGCAGA-3′ and reverse 5′-TAGACATCAGCAGGTTTGCC-3′; *TIMM23* forward 5′-GGATTGAAGGAAACCCAGAA-3′ and reverse 5′-CCCTTGCCTAGTCACCATATT-3′; *TFAM* forward 5′-GCTCAGAACCCAGATGCAA-3′ and reverse 5′-CACTCCGCCCTATAAGCATC-3′; *TIMM9* forward 5′-TGAAGAGACCACCTGTTCAGA-3′ and reverse 5′-AAGGAGTCCTGCTTTGGCT-3′; *TIMM44* forward 5′-TCCAAGACAGAGATGTCGGA-3′ and reverse 5′-GATGTCGTTCTCGCACTGTT-3′; *VDAC1* forward 5′-AATGTGAATGACGGGACAGA-3′ and reverse 5′-ACAGCGGTCTCCAACTTCTT-3′; *NDUFA9* forward 5′-TATGCATCGGTTTGGTCCTA-3′ and reverse 5′-GACCAACGAAAGCAAAGGAT-3′; *NDUFV2* forward 5′-AAAGGCAGAATGGGTGGTT-3′ and reverse 5′-CTTTCCAACTGGCTTTCGAT-3′; *COX5A* forward 5′-GATGCTCGCTGGGTAACATA-3′ and reverse 5′-GGGCTCTGGAACCATATCAT-3′; *NDUFS5* forward 5′-TGGTGAACAGCCCTACAAGA-3′ and reverse 5′-TCTGCCCGAGTATAACCGAT-3′; *NDUFA2* forward 5′-CGTCAGGGACTTCATTGAGA-3′ and reverse 5′-AAGGGACATTCGTCTCTTGG-3′; *PITRM1* forward 5′-TCTCGGATGAGATGAAGCAG-3′ and reverse 5′-CCCAGTGCCGAGGTATCTAT-3′; *CISD1* forward 5′-TGACTTCCAGTTCCAGCGTA-3′ and reverse 5′-GATAACCAATTGCAGCTGTCC-3′; *ATP5G1* forward 5′-AGCTCTGATCCGCTGTTGTA-3′ and reverse 5′-GGAAGTTGCTGTAGGAAGGC-3′; *MRPL12* forward 5′-TCAACGAGCTCCTGAAGAAA-3′ and reverse 5′-GTGTCCGTTCTTTCGCTATG-3′; *IDH3A* forward 5′-CCGACCATGTGTCTCTATCG-3′ and reverse 5′-GCACGACTCCATCAACAATC-3′; *18S* forward 5′-CGCCGCTAGAGGTGAAATTC-3′ and reverse 5′-CTTTCGCTCTGGTCCGTCTT-3′; *PPARGC1A* forward 5′-CTCACACCAAACCCACAGAG-3′ and reverse 5′-GTGTTGTGACTGCGACTGTG-3′; *PEX1* forward 5′-AGTCACCAGCCTGCATTTCTT-3′ and reverse 5′-ATGGGAACATGGCTTGAGAA-3′.

Mouse primers used in RT–qPCR were as follows. Ppargc1a forward 5′-GACAGCTTTCTGGGTGGATT-3′ and reverse 5′-CGCAGGCTCATTGTTGTACT-3′; *Actb* forward 5′-GATGACGATATCGCTGCGCTG-3′ and reverse 5′-GTACGACCAGAGGCATACAGG-3′.

Quantification of mitochondrial DNA content. Total DNA was isolated using the Puregene Blood Core kit (Qiagen) according to the manufacturer's instructions. Mitochondrial DNA content was measured using real-time quantitative PCR (qPCR) as previously described[57]. In brief, relative quantification of mitochondrial DNA content for each sample was determined using a set of mitochondrial specific primers: mt-Mito: forward 5′-CACTTTCCACACAGACATCA-3′, reverse 5′-TGGTTAGGCTGGTGTTAGGG-3′; and a set of nuclear-specific primers: *B2M* forward 5′-TGTTCCTGCTGGGTAGCTCT-3′ and reverse 5′-CCTCCATGATGCTGCTTACA-3′. qPCR was performed with a Roche 480 thermal cycler, using SYBR Green PCR Master Mix (Applied Biosystems). qPCR conditions used were: 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 72 °C for 15 s followed by a melting cycle going up to 95 °C. Primer specificity was determined by melt curve analysis and agarose gel electrophoresis, confirming a single band of the amplification product. The relative mitochondrial DNA content was calculated using the $2^{-\Delta\Delta C_t}$ method where ΔC_t is $C_t^{\text{mt-Mito}} - C_t^{B2M}$.

Mitochondria analysis by flow cytometry. Cells were seeded in 60-mm dishes and cultured in DMEM containing 10% FBS. Cells were washed once with FBS-free medium. Mitotracker Red (LifeTechnologies, M7212) was added to a final concentration of 20–40 nM and incubated for 20–30 min at 37 °C. Cells were then quickly washed with PBS, trypsinized, collected in phenol-red-free medium and incubated for 10 min at 37 °C in the dark before analysis. Unstained cells were used as a negative control. Acquisition was performed on LSR II Flow Cytometer (BD Biosciences) on the basis of forward and sideward scatter parameters and Texas red fluorescence using BD FACSDiva software. Eight to ten thousand events from each sample were evaluated. Data were analysed using the FCS Express 6 Flow software (*De novo* Software).

Metabolic assays. *Measurement of oxygen consumption rate and extracellular acidification rate.* The functional status of mitochondria in cells expressing F3–T3 was determined by analysing multiple parameters of oxidative metabolism using the XF96 Extracellular Flux Analyzer (Agilent), which measures the extracellular flux changes of oxygen and protons. Cells were plated in XF96-well microplates (6,000–7,000 cells per well) in a final volume of 80 μl of DMEM medium (25 mM glucose, 2 mM glutamine) supplemented with 10% FBS, 48 h before the assay. For experiments requiring AZD4547 treatment, cells were plated as previously described in the presence of 150 nM of AZD4547. For the mitochondrial stress test, cells were washed twice with 200 μl of XF Assay Medium Modified DMEM (Agilent), supplemented with 25 mM glucose, 2 mM glutamine (XF-Mito-MEM) and incubated at 37 °C in the absence of CO_2 for 1 h before the assay in 180 μl per well of XF-Mito-MEM. The ports of the sensor cartridge were sequentially loaded with 20 μl per well of the appropriate compound: the ATP coupler oligomycin (Sigma, O4876), the uncoupling agent carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP, Sigma C2920) and the complex I inhibitor rotenone (Sigma, R8875). Compound concentration used for the different cell lines are indicated in Supplementary Table 9a. For the glycolysis stress test, cells were washed twice with