Supporting Information

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SI Materials and Methods

Reagents and Cell Culture. The following antibodies were used in this study and were obtained from Cell Signaling (CS) or Santa Cruz Biotechnology (SC): p-AMPKα (T172) (CS2535), AMPKα (CS2532), p-ACC (S79) (CS3661), ACC (CS3676), p-raptor (S792) (CS2083), raptor (CS2280), p-S6K (CS9206), S6K (CS9202), Pim-1 (SC28777), p-4EBP1 (T37/46) (CS2855), 4EBP1 (CS9452), LKB1 (SC28788), eIF4E (CS9742), mTOR (CS2983), Pim-2 (SC13514), Pim-3 (SC98959), c-Myc (CS9402), PGC-1α (SC13067), eIF4G (CS2498). Additional antibodies were purchased from other sources: actin (Sigma A3854), and GAPDH (Sigma G9295). Mouse embryonic fibroblasts (MEFs), K562, and lung cancer cell lines were cultured in DMEM containing 10% fetal bovine serum.

Construction of Lentiviral Vectors and Preparation of Lentiviral Stocks. Lentiviral shRNA constructs (nontargeting shctl and Pim-3 targeting, shPim-3) were purchased from Open Biosystems. Lentiviral particles were produced by transfecting 293T cells in a 10-cm dish with 9 mg of lentiviral plasmid, 28.5 mg of translentiviral packaging mix (Open Biosystems), and 187.5 mg of transfection reagent, Express-in (Open Biosystems) according to the manufacturer's recommendations. Forty-eight hours after transfection, viral supernatants were collected, filtered, concentrated 25-fold by centrifugation at 50,000 × *g* at 4 °C for 3 h, and aliquots were stored at −80 °C. The titers of the concentrated viral particles were measured with a p24 ELISA kit (Cell Biolabs, Inc.) and found to be between 10^6–10^8 transduction units (TU)/mL. For transduction, 1×10^6 cells were infected with 10^6 TU of viral stock using the Viraductin, lentiviral transduction kit (Cell Biolabs, Inc.) according to the manufacturer's recommendations. Forty-eight hours later, cells were harvested, lysed, and protein expression analyzed by SDS-PAGE followed by Western blotting.

Biochemical Analysis. Relative quantification of gene expression was achieved by quantitative real-time PCR (iQ5 Multicolor Real-Time PCR detection system, BioRad Laboratories) using iQ5 optical system software. The expression levels were normalized to GAPDH. The primers used for real-time PCR are listed below.

Pim-3. Forward TGTGGTCTCTGGGTGTACTGCG
Reverse GACACCACTCAATAAGCTGCTGG

PGC-1α. Forward GAATCAAGCCACTACAGACACCG
Reverse CATCCCTCTTGAGCCTTTCGTG

GAPDH. Forward CCCACTAACATCAAATGGGG
Reverse ATCCACAGTCTTCTGGGTGG

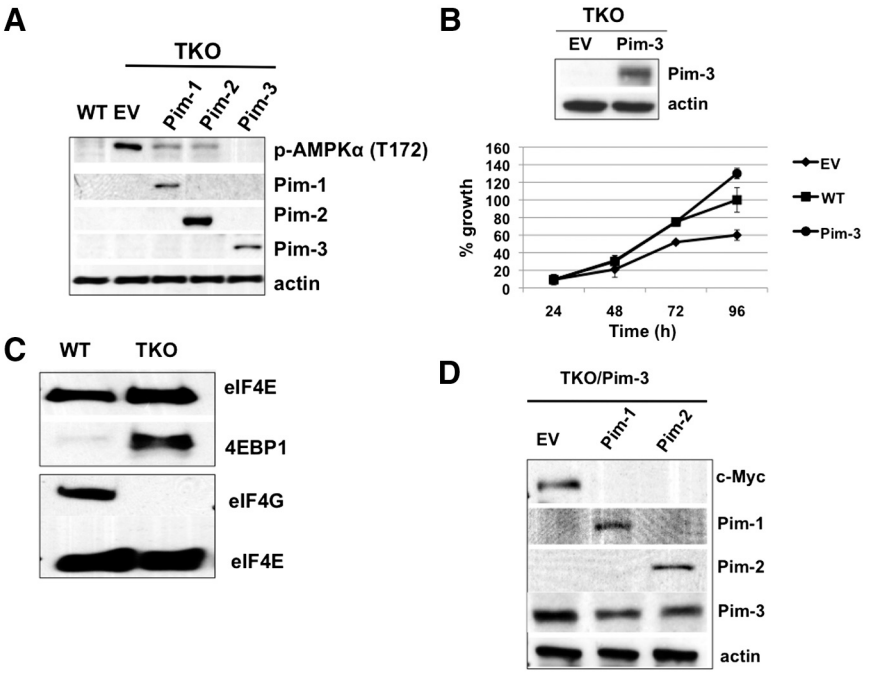

Fig. S1. (*A*) Lysates were prepared 48 h after transduction with empty vector (EV), Pim-1, -2, or -3 lentiviruses and probed for the indicated proteins. The levels in wild-type MEFs (WT) are shown for comparison. (*B*) Expression of Pim-3 in stable cell lines used for growth curve analysis (*Upper*). Growth curve of TKO MEFs infected with EV or Pim-3 as determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Percentage values are relative to the value of WT MEFs at the 96 h time point (100%). The data points are the average of three independent measurements, and the standard deviation from the mean is shown. (*C*) 4EBP1 binding to eIF4E is increased, while eIF4G binding to eIF4E is decreased in TKO MEFs. eIF4E was captured on m^7-GTP resin from WT and TKO MEFs lysate, and the levels of bound 4EBP1 and eIF4G were determined by Western blotting. (*D*) TKO/Pim-3 MEFs were infected with EV, Pim-1, or Pim-2 lentiviruses, and 48 h later lysates were probed for c-Myc and Pim kinase levels.

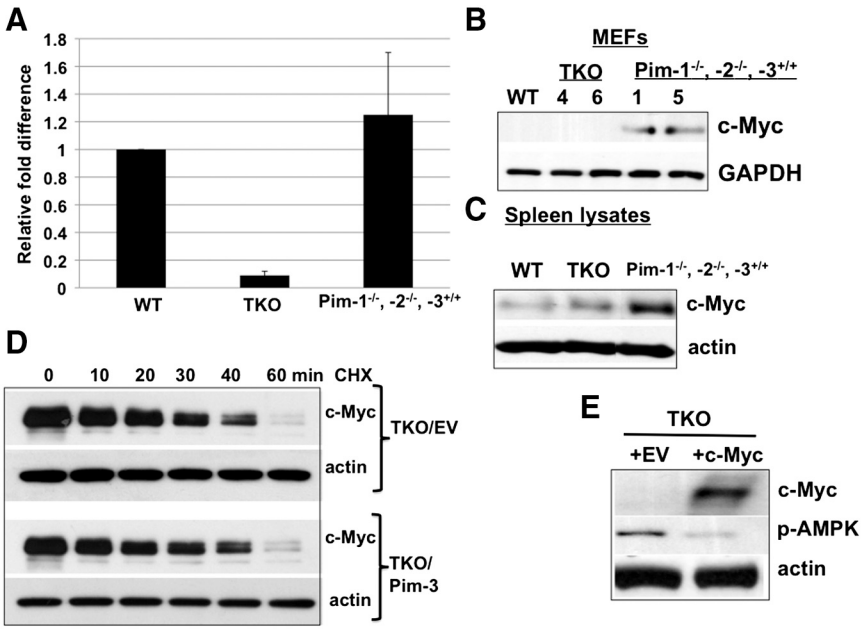

Fig. S2. (*A*) Pim-3 mRNA levels in each of the MEFs determined by quantitative RT-PCR. Values are the average of three independent measurements, and the standard deviation from the mean is shown. (*B*) c-Myc protein levels in WT, TKO, and Pim-3–only MEFs as determined by Western blotting with GAPDH as a loading control. MEFs were generated as described in *Materials and Methods* and were derived from separate embryos. These MEFs were not used for the experiments shown in the main text and only serve to validate that the increased c-Myc protein levels in the Pim-$1^{-/-}$, -$2^{-/-}$, -$3^{+/+}$ cells are not unique to a single MEF cell line. (*C*) Spleen lysates from age-matched WT and TKO mice were probed for c-Myc protein. (*D*) c-Myc protein stability in TKO MEFs expressing empty vector (EV) or Pim-3. Cells were treated with cycloheximide (CHX, 10 μM) and lysates prepared at the indicated time points and probed for c-Myc protein levels. To obtain a relatively equal amount of c-Myc protein at the 0 min time point, ~2.5-fold more TKO/EV protein lysate was loaded relative to TKO/Pim-3. (*E*) Lysates were prepared after 48 h transduction with EV or c-Myc lentiviruses and probed for the indicated proteins.

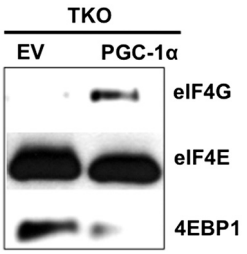

Fig. S3. TKO MEFs were infected with empty vector (EV) or PGC-1α lentiviruses, and 48 h later eIF4E was captured on m^7-GTP resin and binding to eIF4G and 4EBP1 was determined.