SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Pim1, Pim2, Pim3 and K-Ras^{G12V} expression in MEFs.

(**A**) Pim mRNA levels in MEF cells. Reverse transcriptase (RT)-PCR analysis for Pim1, Pim2 and Pim3 expression in MEF cells including wild type (WT), Pim1, 2, 3 triple knockout (TKO) and Pim1, 3 double knockout (DKO). (**B**) Growth curve of MEF cells. Cell number was determined by crystal violet staining assay (mean+/- SD, n=4). (**C**) K-Ras^{G12V} expression in MEFs. Immunoblot analysis of K-Ras, p-ERK1/2 and ERK1/2 protein expression. WT and TKO MEF cells were transduced with empty vector (EV) or K-Ras^{G12V} for three days and then their lysates were used for SDS-PAGE. (**D**) The generation of Pim single knockout MEF cells. Pim knockout MEF cells were obtained from Pim1, Pim2, or Pim3 single KO embryos. RT-PCR analysis was used for the measurement of Pim1, 2 and 3 expression in MEF cells. (**E**) Reconstitution of Pim1, 2, or 3 expression in TKO MEFs. RT-PCR analysis of Pim1, 2, or 3 expression in TKO MEFs using an red fluorescent protein control vector (empty vector), Pim1, Pim2, Pim3 or all three Pim containing vectors.

Figure S2. Accumulation of reactive oxygen species (ROS) by loss of Pim kianses.

(**A**) ROS levels in WT and TKO MEFs. MEFs were co-stained with CM-H_2DCFDA (DCF; green) and MitoTracker (red). (**B**) Flow cytometric analysis of DCF fluorescence in WT and TKO MEF cells. A non-stained control (N.C.) of WT MEFs has been included. (**C**) Mitochondrial and lipid ROS production in MEF cells was assessed by flow cytometry using MitoSOX (5 μM) and C11-BODIPY (2 μM). (**D**) Lipid peroxide levels as measured by the amount of thiobarbituric acid-reactive substances (TBARS). The lipid peroxide concentration is expressed as nanomoles per milligram protein using malondialdehyde as a reference standard

(Mean +/- SD, triplicates). (**E**) Detection of ROS induced by K-Ras^{G12V} in Pim1$^{-/-}$, Pim2$^{-/-}$, and Pim3$^{-/-}$ MEF cells. (**F**) Cell death induced by KRAS was determined by a trypan blue exclusion (Mean +/- SD, triplicates). TKO MEF cells expressing empty vector (EV) or KRAS were treated with DMSO (or PBS), L-NMMA (10 μM), DPI (10 μM), PEG-superoxide dismutase (SOD; 400 U/mL), PEG-catalase (CAT; 800 U/mL), or SOD plus CAT for 48 hours. (G) Morphological changes seen with cell death induction. (**H**) ROS induction by pan-Pim inhibitors. WT MEF cells were treated with DMSO, 5 μM of GNE-652, or NVP-LGB321 for 24 hours and then stained with 1 μM of DCF for 30 min prior to carrying out flow cytometric analysis. (**I**) Fluorescence microscopy to detect ROS detection. (**J**) Cell viability was determined by MTT assay. WT MEF cells were treated with DMSO, GNE652, or NVP-LGB321 at the dose indicated for 48 hours in presence of either PBS or 5 mM NAC (mean +/- SD, n=4). * $p<0.05$. (**K**) Changes in NSCLC cell viability and cell death after GNE-652 treatment for 72 hours (Mean +/- SD, n=4). (**L**) Morphological cell death of NSCLC cells.

Figure S3. Gene set enrichment analysis demonstrates a correlation between cell metabolic pathways and Pim kinase deletion.

(**A**) GSEA was performed using gene sets from the Molecular Signatures Database for curated gene sets (MSigDB-C2 v2, BROAD Institute). In order to avoid false positives due to multiple testing in GSEA, the FDR is used to adjust the *P*-value to give the *q*-value. A *q*-value of <0.05 is statistically significant. WT, wild type; KN, knockout of triple Pims. (**B**) Expression of key glycolytic enzymes is repressed in kidney tissue of TKO mice. Immunoblot analysis was performed for glycolytic enzyme expression in the kidney tissues isolated from WT and TKO mice. Anti-Hk2, Pkm1/2, Pfk-1, Enolase, Ldha, Gapdh and β-actin antibodies were evaluated.

Figure S4. Loss of Pim kinases represses glycolytic activity.

Loss of Pim kinases represses glycolytic activity in splenic T cells. (**A**) Splenocytes from either wild type (WT) or TKO mice were stimulated with plate bound anti-CD3 and anti-CD28 (5µg/ml each) in culture media. RNA was prepared from the cell pellets and expression of the genes was measured. (**B**) Splenocytes from either wild type (WT) or TKO mice were first labeled with CFSE and stimulated with plate bound anti-CD3 and anti-CD28 (5µg/ml each) in culture media. Proliferation was checked after 48h by FACS and the data was analyzed using FlowJo software. The data shown is representative of three experiments with similar results.

Figure S5. Mitochondrial bioenergetics and metabolism are influenced by loss of Pim kinases.

(**A**) Oxygen consumption rate (OCR) was determined by Seahorse XF24 assay. TKO MEF cells were transduced with empty vector (EV), Pim-1, -2 or -3. (**B**) Expression mitochondrial metabolism genes, *Cox2, Cox5a, Cytb, cytc, Gabpa, Mcad, Pgc1a, Pgc1b, Pprc1, Atp5g1*, and *Ucp2,* was determined by qPCR analysis. (**C**) qPCR analysis of mitochondrial gene expression.

Figure S6. Pim kinases regulates NAD(P)$^+$, Sod enzymes and Prdx3 levels.

(**A**) Levels of nicotinamide metabolic intermediates. Levels of nicotinamide, NAD^+, $NADP^+$, NADH, NMN, nicotinamide riboside, $NAAD^+$ in WT and TKO MEFs are shown (Mean +/- SD, n=5). The results of glucose depletion for 16 h are shown. (**B**) The ratio of $NADP^+$/NADPH ratio was determined by enzymatic assay (see Methods) (Mean +/- SD, n=3). (**C**) Expression

levels of Gpx4 mRNA from the bead array and protein by western blot. (**D**) Expression of *Sod1, Sod2, Sod3*, and *Prdx3* mRNA normalized to *β-Actin* mRNA levels measured by qPCR (mean +/- SD, triplicates). Whole cell extracts were isolated from MEF cells, and immunoblotted with antibodies to anti-Sod2 and β-actin proteins. (**E**) SOD enzyme activity (mean +/- SD, triplicates). (**F**) q-PCR analysis for mitochondrial DNA content (the ratio of mitochondrially encoded *Cox2* to an intron of the nuclear-encoded *β-globin* gene). (**G**) DCF-DA staining for detection of ROS. (**H**) Western blot analysis for c-Myc expression. c-Myc was transduced into TKO or WT cells and 48 h later cell extracts were probed with antibodies as shown. β-actin functions as a loading control.

SUPPLEMENTAL TABLE

Table S1. Loss of Pim kinases causes downregulation of gene expression controlling specific metabolic processes (GSEA).

Figure S1

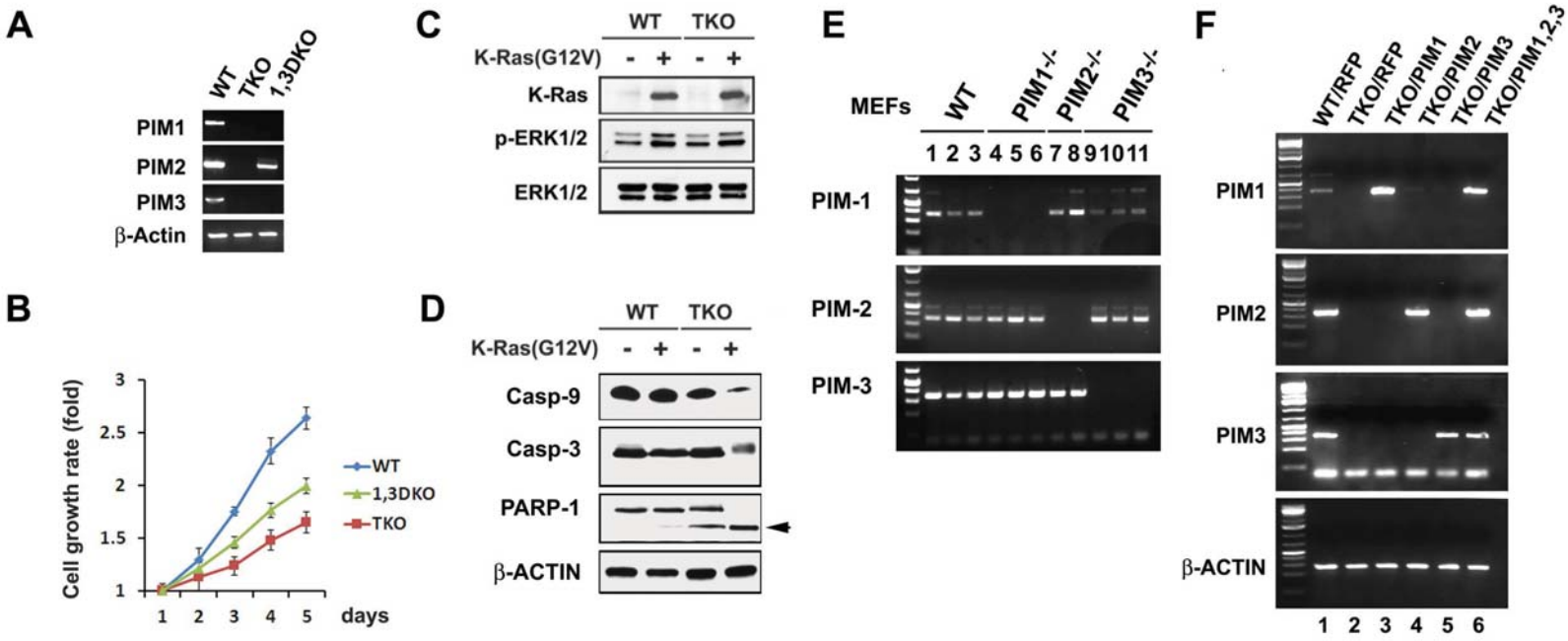

A
WT
TKO
1,3DKO
PIM1
PIM2
PIM3
β-Actin
B
Cell growth rate (fold)
3
2.5
2
1.5
1
1 2 3 4 5
days
WT
1,3DKO
TKO
C
WT
TKO
K-Ras(G12V) - + - +
K-Ras
p-ERK1/2
ERK1/2
D
WT
TKO
K-Ras(G12V) - + - +
Casp-9
Casp-3
PARP-1
β-ACTIN
E
MEFs
WT
PIM1-/-
PIM2-/-
PIM3-/-
1 2 3 4 5 6 7 8 9 10 11
PIM-1
PIM-2
PIM-3
F
WT/RFP
TKO/RFP
TKO/PIM1
TKO/PIM2
TKO/PIM3
TKO/PIM1,2,3
PIM1
PIM2
PIM3
β-ACTIN
1 2 3 4 5 6

Figure S2

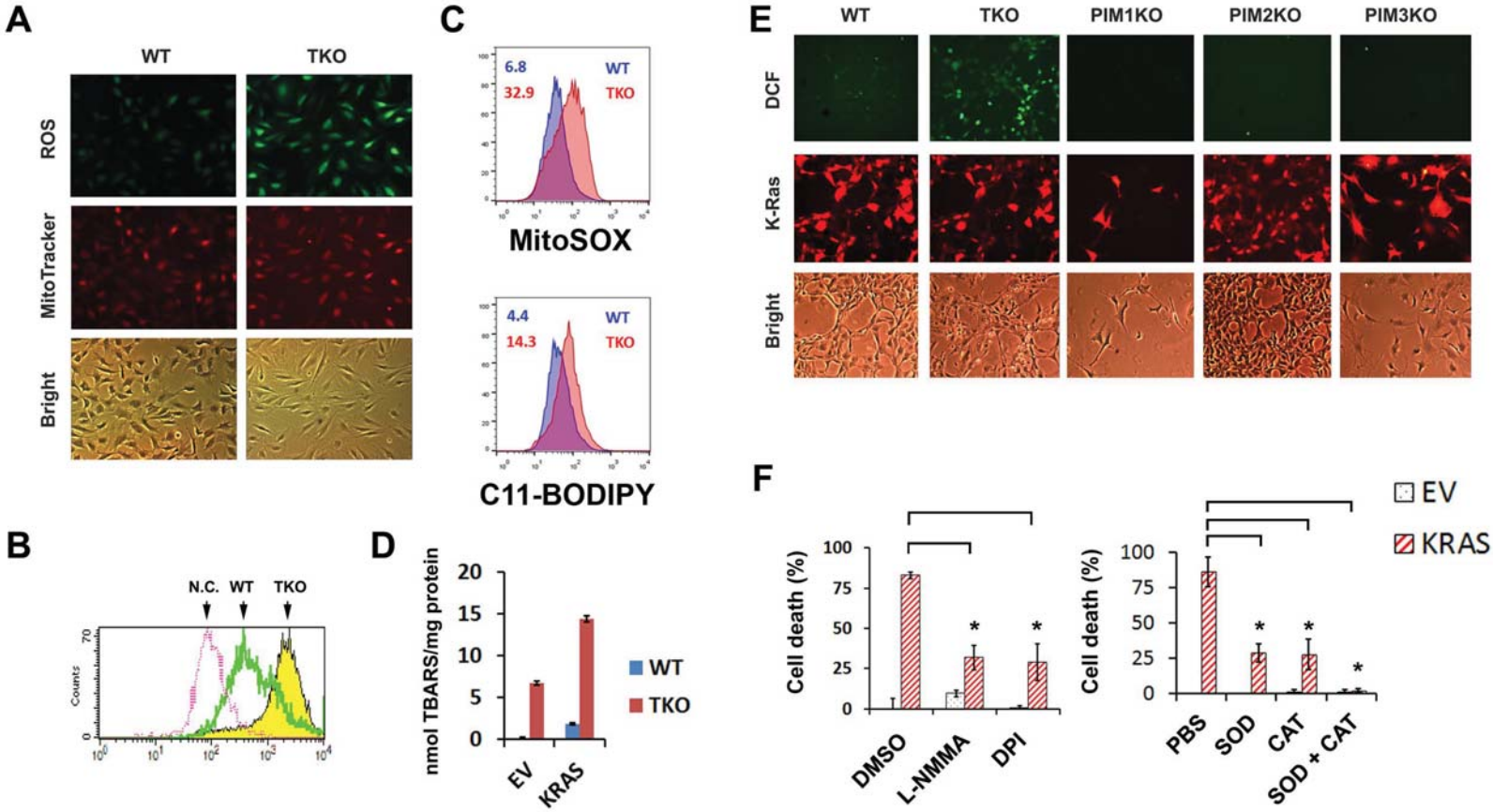

A
WT
TKO
ROS
MitoTracker
Bright
C
6.8
32.9
WT
TKO
MitoSOX
4.4
14.3
WT
TKO
C11-BODIPY
E
WT
TKO
PIM1KO
PIM2KO
PIM3KO
DCF
K-Ras
Bright
B
N.C.
WT
TKO
Counts
D
nmol TBARS/mg protein
WT
TKO
EV
KRAS
F
EV
KRAS
Cell death (%)
DMSO
L-NMMA
DPI
PBS
SOD
CAT
SOD + CAT

Figure S2

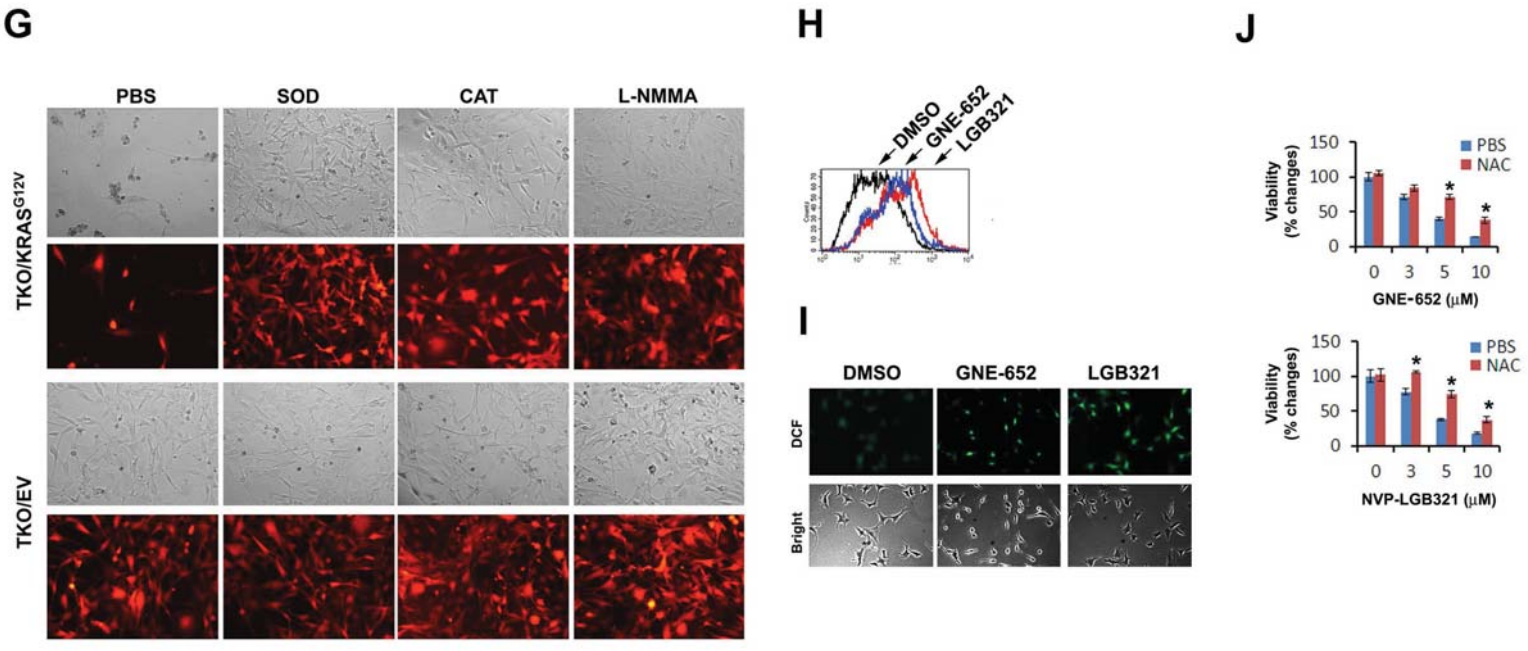

G
PBS
SOD
CAT
L-NMMA
TKO/KRASG12V
TKO/EV
H
DMSO
GNE-652
LGB321
I
DMSO
GNE-652
LGB321
DCF
Bright
J
Viability (% changes)
PBS
NAC
GNE-652 (μM)
NVP-LGB321 (μM)

Figure S2

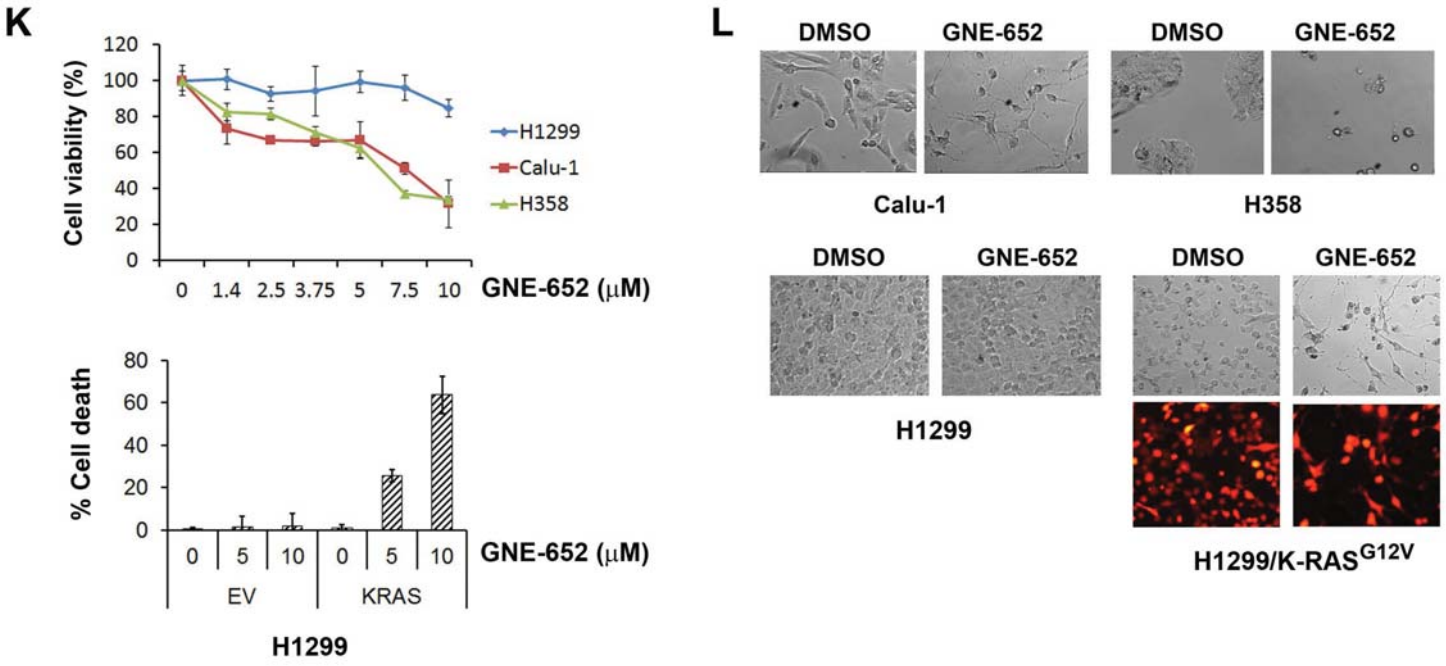

K
Cell viability (%)
120
100
80
60
40
20
0
H1299
Calu-1
H358
0 1.4 2.5 3.75 5 7.5 10
GNE-652 (μM)
% Cell death
80
60
40
20
0
0 5 10 0 5 10
GNE-652 (μM)
EV
KRAS
H1299
L
DMSO
GNE-652
DMSO
GNE-652
Calu-1
H358
DMSO
GNE-652
DMSO
GNE-652
H1299
H1299/K-RASG12V

Figure S3

A

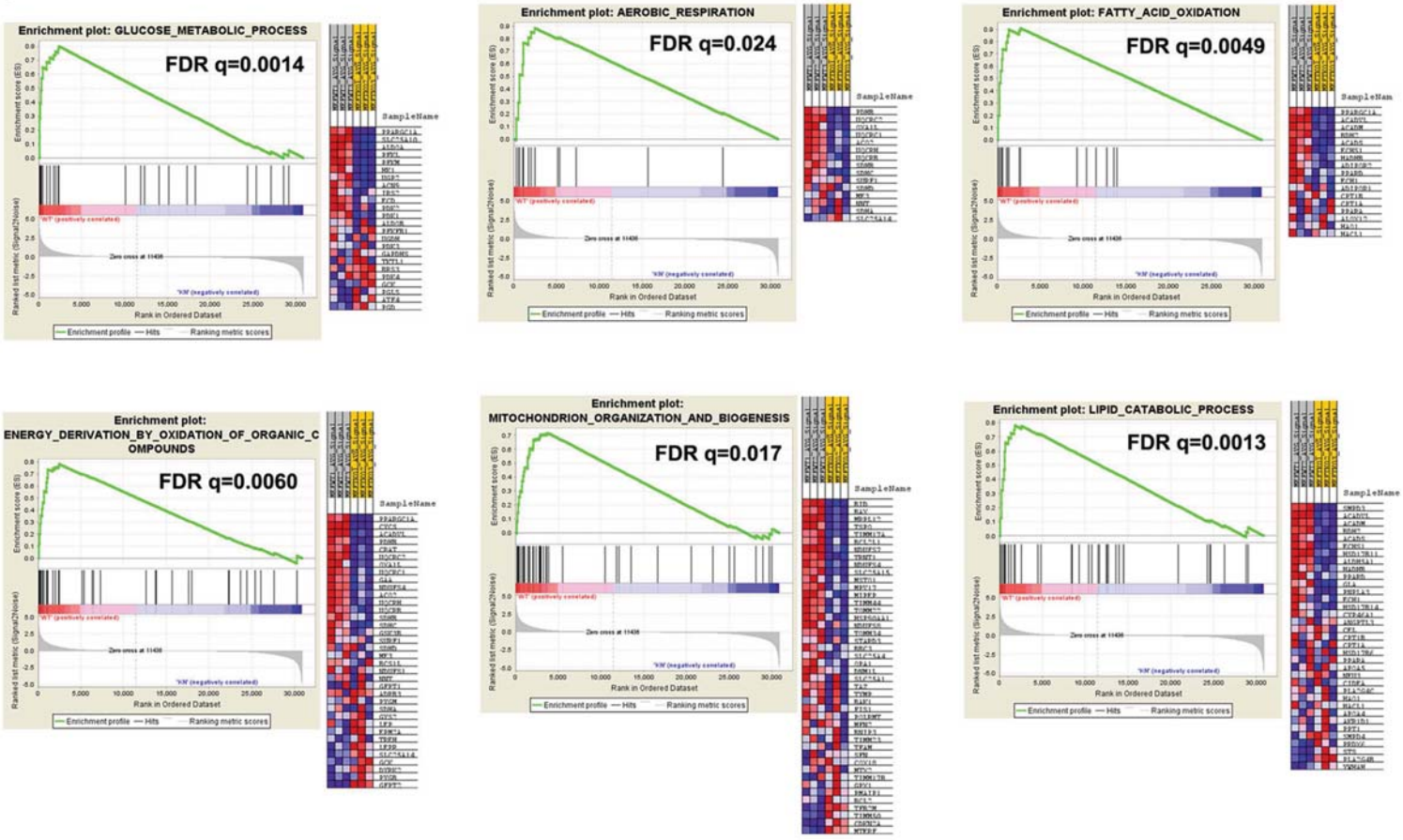
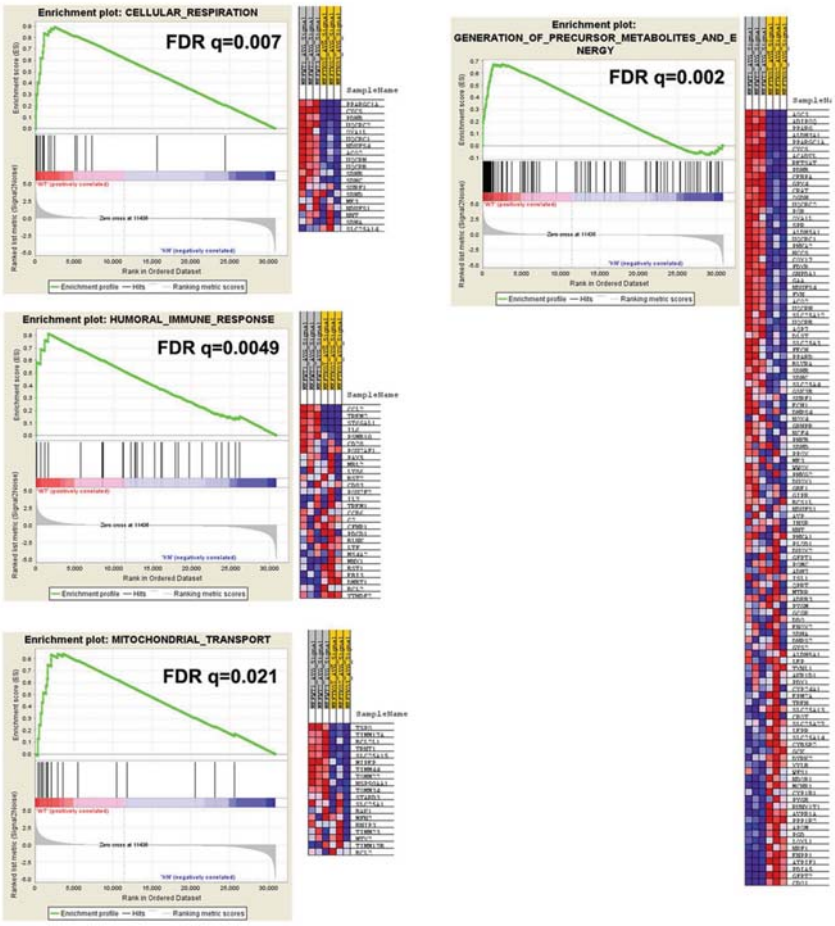

Enrichment plot: GLUCOSE_METABOLIC_PROCESS
FDR q=0.0014
Enrichment plot: AEROBIC_RESPIRATION
FDR q=0.024
Enrichment plot: FATTY_ACID_OXIDATION
FDR q=0.0049
Enrichment plot: ENERGY_DERIVATION_BY_OXIDATION_OF_ORGANIC_COMPOUNDS
FDR q=0.0060
Enrichment plot: MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS
FDR q=0.017
Enrichment plot: LIPID_CATABOLIC_PROCESS
FDR q=0.0013
Enrichment score (ES)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
SampleName
Enrichment profile
Hits
Ranking metric scores

Figure S3

A


Enrichment plot: CELLULAR_RESPIRATION
FDR q=0.007
Enrichment plot: HUMORAL_IMMUNE_RESPONSE
FDR q=0.0049
Enrichment plot: MITOCHONDRIAL_TRANSPORT
FDR q=0.021
Enrichment plot: GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY
FDR q=0.002
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

B

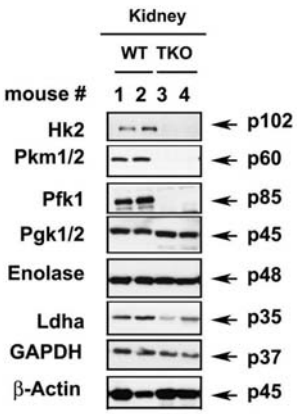

Kidney
WT TKO
mouse # 1 2 3 4
Hk2
p102
Pkm1/2
p60
Pfk1
p85
Pgk1/2
p45
Enolase
p48
Ldha
p35
GAPDH
p37
β-Actin
p45

Figure S4

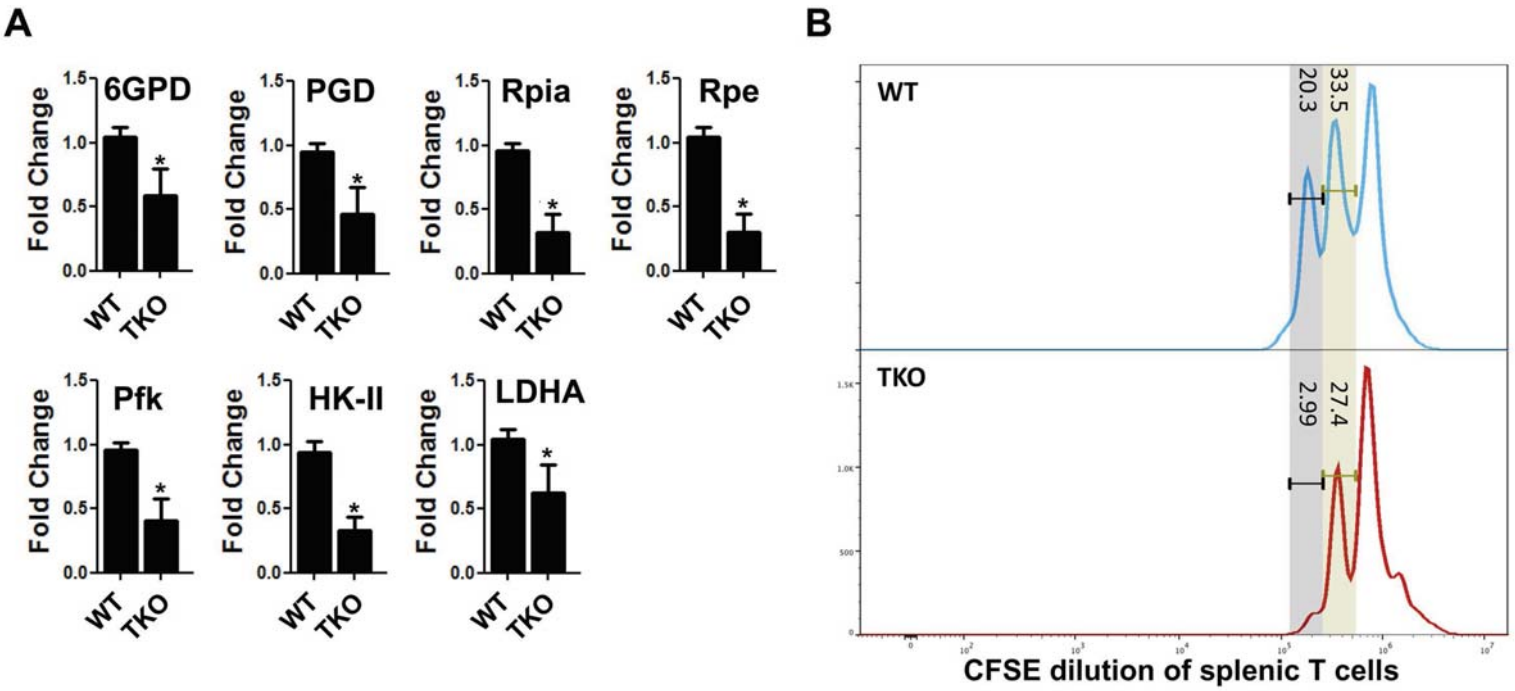

A
6GPD
PGD
Rpia
Rpe
Pfk
HK-II
LDHA
Fold Change
1.5
1.0
0.5
0.0
WT
TKO
*
B
WT
TKO
20.3
33.5
2.99
27.4
CFSE dilution of splenic T cells

Figure S5

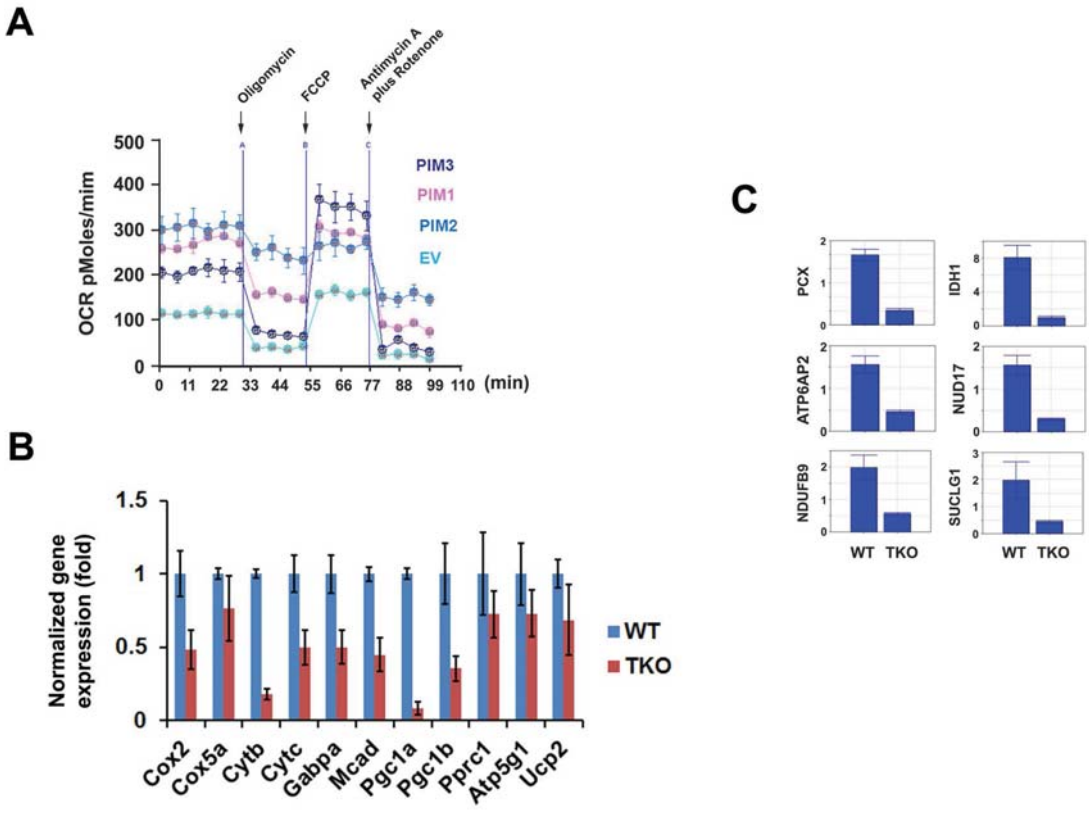

A
Oligomycin
FCCP
Antimycin A plus Rotenone
OCR pMoles/mim
500
400
300
200
100
0
0 11 22 33 44 55 66 77 88 99 110 (min)
PIM3
PIM1
PIM2
EV
B
Normalized gene expression (fold)
1.5
1
0.5
0
Cox2
Cox5a
Cytb
Cytc
Gabpa
Mcad
Pgc1a
Pgc1b
Pprc1
Atp5g1
Ucp2
WT
TKO
C
PCX
IDH1
ATP6AP2
NUD17
NDUFB9
SUCLG1
WT TKO
WT TKO

Figure S6

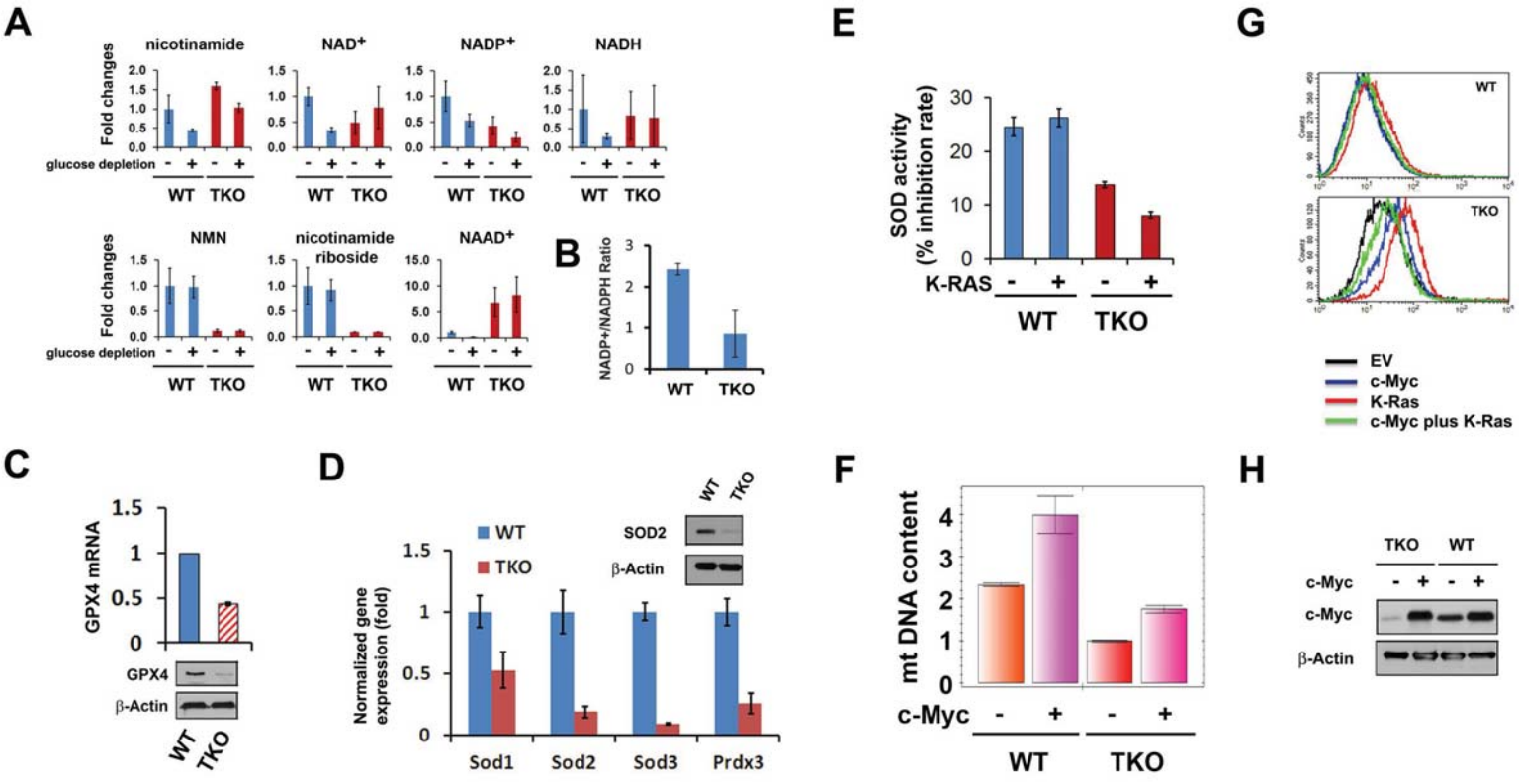

A
nicotinamide
NAD+
NADP+
NADH
Fold changes
glucose depletion
WT
TKO
NMN
nicotinamide riboside
NAAD+
B
NADP+/NADPH Ratio
C
GPX4 mRNA
GPX4
β-Actin
D
WT
TKO
SOD2
β-Actin
Normalized gene expression (fold)
Sod1
Sod2
Sod3
Prdx3
E
SOD activity (% inhibition rate)
K-RAS
F
mt DNA content
c-Myc
G
EV
c-Myc
K-Ras
c-Myc plus K-Ras
H
c-Myc
β-Actin

Table S1. Loss of Pim kinases causes downregulation of gene expression controlling specific metabolic processes.

Gene Set	Total Genes	Genes * Found	FDR q-value	NES
Glucose metabolic process	24	12	0.001	1.76
Aerobic respiration	15	10	0.024	1.72
Cellular respiration	19	13	0.007	1.80
Generation of precursor metabolites and energy	112	37	0.002	1.90
Energy derivation by oxidation of organic compounds	36	17	0.006	1.85
Mitochondrion organization and biogenesis	44	28	0.017	1.75
Mitochondrial transport	19	12	0.021	1.73
Fatty acid oxidation	17	11	0.005	1.84
Lipid catabolic process	35	12	0.001	1.77
Humoral immune response	28	5	0.005	1.83

Gene expression arrays identified over 1200 genes with significantly altered expression levels following loss of Pim kinases (TKO MEFs) compared to control WT MEFs. GSEA was used to determine gene sets significantly altered by loss of Pim kinases. Selected metabolic gene sets are shown. NES, normalised enrichment score; *q*-values, FDR-adjusted *P*-value. * denotes numbers of core enrichment genes.

SUPPLEMENTAL METHODS AND MATERIALS

Maintenance of mouse colonies and generation of MEFs.

All animals were kept in the knockout mouse facility at the Hollings Cancer Center according to the IACUC approval in Medical University of South Carolina. MEFs were generated from mouse embryos (day 14.5) of Friend virus B wild-type (WT) mice and genetically modified mice deleted in *Pim-1*, *-2* and *-3*. Because TKO mice were infertile, we crossed a male $Pim1^{+/-}$, 2,3DKO mouse ($Pim1^{+/-}$; $Pim2^{-/-}$; $Pim3^{-/-}$) with a female $Pim2^{+/-}$,1,3DKO mouse ($Pim1^{-/-}$; $Pim2^{+/-}$; $Pim3^{-/-}$) to obtain TKO ($Pim1^{-/-}$; $Pim2^{-/-}$; $Pim3^{-/-}$) embryos. Embryo tissues (after surgically removing head and organs) were trypsinized for 3 min. The adherent cells were initially grown in Dulbecco's modified Eagle's medium (Hyclone) supplemented with 10% newborn calf serum (Sigma; N4637), 1% Glutamax (Gibco) and 1 % Pen Strep (Gibco) in 175 cm^2 tissue culture flasks. When primary MEF cells were confluent, these cells were harvested and reseeded at the density of $3x10^5$ cells into 75 cm^2 tissue culture flask and grown in DMEM supplemented with 10% fetal bovine serum, 1% Glutamax and 1 % Pen Strep. Every three days, cells were counted and reseeded at the density of $3x10^5$ cells until MEF cells were immortalized.

Plasmids

Full-length cDNAs encoding mouse K-Ras (G12V) and the control vector FUCRW (red fluorescent protein marker) were kind gifts of Drs. Owen Witte and Houjian Cai (University of California, Los Angeles).[1] Pim-1, Pim-2, and Pim-3 were cloned into FUCRW vector. An HA-tagged c-Myc was subcloned into pLEX vector (Open Biosystems) as reported previously. [2] Lentiviruses in the FUCRW vector expressing K-Ras, Pim-1, Pim-2, and Pim-3 were prepared

according to the protocol reported by Goldstein AS *et al.*[3] Lentiviruses containing pLEX vector expressing HA-tagged c-Myc were prepared according to the manufacturer's' protocol (Open Biosystems). [4]

Measurements of reactive oxygen species (ROS)

For the determination of oxidative stress, cells were washed once with PBS and incubated with PBS containing 2 μM CM-H_2DCFDA and 100 nM MitoTracker Red FM (Life Technologies) for 30 min at 37 °C. For detection of mitochondrial superoxide anion and lipid ROS, 5 μM MitoSOX and 2 μM C11-BODIPY (Life Technologies) were used. For confocal microscopy, live cells were imaged using a confocal fluorescence microscope (FV10i Laser Scanning Confocal, Olympus) and Nikon fluorescence microscope (Eclipse TE2000-S) equipped with X-Cite™ 120 Fluroescence Illumination system (EXFO). For quantitative comparison of cellular ROS levels between WT and TKO MEFs, DCF-labelled cells were analyzed by flow cytometry (BD FACSCalibur).

Measurements of lipid peroxides

Lipid peroxides were assessed by the measurement of malondialdehyde (MDA) concentration using the thiobarbituric acid-reactive substances (TBARS) method according to the manufacturer's protocol (Sigma-Aldrich; MAK085). The MDA concentration is expressed as nanomoles per milligram protein.

Measurements of superoxide dismutase (SOD) enzyme activity

Total SOD enzyme activity was assayed with a Superoxide Dismutase Activity Colorimetric Assay Kit kit (Abcam; ab65354) according to the manufacturer's protocol. Data was normalized with protein content for each sample.

Measurements of glutathione content

Total glutathione (GSH + GSSG), GSSG, and GSH/GSSG ratios were assayed with a GSH/GSSG-Glo assay kit (Promega; V6611) according to the manufacturer's protocol. Data was normalized with protein content for each sample.

Measurements of NADH and NADPH content

Total NADP (NADP and NADPH) and NADPH were quantified using a NAD/NADH Quantification Kit (Sigma; MAK037) and a NADP/NADPH Quantification Kit (Sigma; MAK038) according to the manufacturer's protocols. Data was normalized with the protein content for each sample.

Measurements of ATP content

Aliquot of cells was applied to measurement of cellular ATP using a Luminescent ATP Detection Assay Kit (Abcam; ab113849) according to the manufacturer's protocol.

Measurements of oxygen consumption rate

Oxygen consumption rates were measured with an XF-24 extracellular flux analyzer (SeaHorse Bioscience Inc., Chicopee, MA). To assess mitochondrial function, the XF Cell Mito Stress Test Kit (101706-100) was employed. WT MEF cells were seeded on XF24 V7 plates at

the density of 3 x 10^5 cells/well in 1 ml of DMEM medium. Considering the slow growth of TKO cells, 5 x 10^5 cells of TKO were placed in 1 ml of DMEM medium. After 16 hours, the culture medium was replaced with 0.7 ml of XF assay medium supplemented with 25 mM glucose and 1% glutamax. OCR was measured for 3 min periods with 5 intervals between measurements. After baseline measurements, oligomycin, FCCP, or the combination of Rotenone and antimycin A was sequentially added. Numbers of cells were counted at the end of the experiments.

Colony forming assay

Cells were plated in 1 ml of DMEM on a 6-well plates at a density of 500 cells and then infected with lentiviruses containing empty vector, K-RAS^{G12V}, or c-Myc. Infected cells were cultured at 37 °C for 10–14 days before scoring for colony formation. Colonies were visualized with crystal violet staining.

Metabolic profiling

Immortalized WT and TKO MEF cells were maintained in glucose-free, serum-free, or full growth medium for 16 hours. Cell pellets (200 μL volume) were collected individually as 5 replicates for each test group and stored at -80 °C. Global metabolite analysis was performed by Metabolon, Inc. (Durham, NC) using a combination of ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; positive mode), UPLC-MS/MS (negative mode), and gas chromatography–mass spectrometry (GC-MS) as described previously.[5,6] For both UPLC-MS/MS and GC–MS, spectral files were searched using metabolomic

libraries created by Metabolon that contain approximately 800 commercially available compounds.

Subcellular fractionation

The cells were harvested and the cell pellet was suspended in 5 volumes of isotonic buffer (10 mM HEPES-KOH [pH 7.5], 210 mM mannitol, 70 mM sucrose, 1 mM Na-EDTA, and 1 mM Na-EGTA) supplemented with 1 mM phenylmethylsulfonyl fluoride and protease inhibitor mixture. The cells were incubated on ice for 15 minutes and passed through a 22-gauge needle 15 times. After centrifugation twice at 700g for 10 min. at 4°C, the supernatant was collected and centrifuged at 13,000g for 10 minutes at 4°C. The supernatant was gently transferred and labeled as the cytosolic fraction after one more centrifugation (13,000g for 10 min at 4°C). The pellets were washed with isotonic buffer and then suspended in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton-X 100, 1 mM PMSF, and 0.2% protease inhibitor cocktail), centrifuged (16,000g for 10 min at 4°C), and the resulting supernatants were designated as the mitochondrial fractions. The protein concentrations in cytosolic and mitochondrial fractions were determined by the Bradford assay (Bio-Rad) and equal amounts of mitochondrial and cytosolic proteins were subjected to immunoblotting analysis using β-actin antibody (Sigma) as a loading control. Immunoblotted membranes were also stained with Ponceau S (BP103-10, Fisher) to verify equal protein loading.

Carboxyfluorescein succinimidyl ester (CFSE) based proliferation assay

Splenic T cells were labeled with 1.0 μM CFSE (Molecular Probes, Carlsbad, CA) and subsequently stimulated using anti-CD3 and anti-CD28 antibody (each 5 μg/ml). Cells were

acquired using FACS Calibur (BD Bioscience) and the data was analyzed using FlowJo Software (Oregon, WA) to determine the progressive halving of CFSE fluorescence within daughter cells following each cell division.

Immunoblot analysis

Cells and mouse tissues were washed with PBS without calcium and magnesium and then lysed in RIPA lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, 0.1%SDS, and 1% NP40) with protease and phosphatase inhibitors and 25–50 μg protein loaded on SDS-PAGE gels. Immunoblots were probed with the following antibodies: Kras (sc-30), Glut1 (sc-7903), Hk2 (sc-28889), Pkm1/2 (sc-3106), Pfk1 (sc-67028), Pgk1/2 (sc-28784), Enolase (sc-15343), Sirt1 (sc-15404), purchased from Santa Cruz Biotechnologies; c-Myc (9402), ERK1/2 (9102), p-ERK1/2 (4370), HA (3724), Ldha (2102), purchased from Cell Signaling; SOD2 (ab13533), OxPhos antibody cocktail (ab110413), purchased from Abcam; GAPDH (G9295) and β-actin (A-3854), purchased from Sigma.

RNA preparation, reverse transcription (RT) PCR, quantitative real-time (qRT) PCR, and array analysis

Total RNA was extracted using Trizol (Invitrogen) and RNeasy kits (Qiagen). Total RNA (1–5 μg) was used for first-strand cDNA synthesis using oligo-dT primers and SuperScript II reverse transcriptase (Invitrogen). Real-time PCR was performed with SYBR Green PCR master mix (Bio-Rad) in 96-well plates in a Bio-Rad (Bio-Rad) using Bio-Rad iQ5 real-time PCR detection system. All primers used were from the IDT Integrated Technologies. The relative amount of mRNA was calculated using the comparative CT method after normalization to β-

actin. Gene expression array analysis was performed by the Medical University of South Carolina Gene Core facility Service using Illumina Bead-Array Chip (MouseWG-6 v2.0 Expression BeadChips). c-Myc enrichment analysis was serviced through the HHMI/Keck Biotechnology Resource Laboratory Service (Yale University).

Quantitative analysis of mitochondrial DNA content by qPCR

To assess mitochondrial DNA content in MEF cells, genomic DNA was extracted using the Isolate II Genomic DNA kit (Bio-52065, Bioline). This genomic DNA (2ng) was used for qPCR. Mitochondrial DNA content was determined by measuring the ratio of mitochondrially encoded Cox2 to an intron in the nuclear-encoded beta-globin gene. Similarly, the ratio of mitochondrial cytochrome b to an intron of nuclear glucagon was determined.

Quantitative real time PCR primers

Sod1	NM_011434	Forward	TGT GTC CAT TGA AGA TCG TGT G
		Reverse	TCC CAG CAT TTC CAG TCT TTG
Sod2	NM_013671	Forward	TGC TCT AAT CAG GAC CCA TTG
		Reverse	CAT TCT CCC AGT TGA TTA CAT TCC
Sod3	NM_011435	Forward	TGT CAC CAT GTC AAA TCC AGG
		Reverse	ATC CAG ATC TCC AGC ACT TTG
Prdx3	NM_007452	Forward	TCC CAC TTC AGT CAT CTT GC
		Reverse	TTT CCA ACA GCA CTC CGT AG
Aldoa	NM_007438	Forward	CCC CAA GTT ATC AAG TCC AAG G
		Reverse	GTT CAG ACA GCC CAT CCA G
Pfkm	NM_021514.2	Forward	GAT GGC TTT GAG GGT CTG G
		Reverse	CTT GGT TAT GTT GGC ACT GAT C
Eno3	NM_007933.2	Forward	CTT CAT CCA GAA CTA TCC CGT G
		Reverse	GAG GTC ATC TCC CAC AAT CTG

Agpat9	NM_172715.3	Forward	ATA ACA AGC AGT ACA GAC CCC
		Reverse	ATC AAT CCA CCG TGA ACC TG
Idh1	NM_010497.2	Forward	CCC AAG CTA TGA AGT CCG AG
		Reverse	GCT TCT ACC GTC TTA CCA TCT G
Tgm2	NM_009373.3	Forward	CAC TTT CCG ATC CCC TGT ATG
		Reverse	CCA ATA TCA GTC GGG AAC AGG
Pfkfb2	NM_008825.3	Forward	CTA ACA CGC TAC CTC AAC TGG
		Reverse	GCG GAT CTT CAT AGC CTC TTC
Atp6ap2	NM_027439.3	Forward	TGG TCG TTC TCC TGT TCT TTC
		Reverse	ACC CTG GCG ATC TTA ATA TGC
Nudt7	NM_024437.1	Forward	AAT TCT CTG TCC TTG TAC CAC TG
		Reverse	TGG CTG TGT CAT CTG TGT C
Pcx	NM_008797.2	Forward	GAG CTT ATC CCG AAC ATC CC
		Reverse	TCC ATA CCA TTC TCT TTG GCC
Suclg1	NM_019879.2	Forward	AAT GAT CCA GCC ACA GAA GG
		Reverse	AGC AAT GAA GGA CAC TAC AGG
Hk1	NM_010438.2	Forward	TCA CAT TGT CTC CTG CAT CTC
		Reverse	CTT TGA ATC CCT TTG TCC ACG
Ndufb9	NM_023172.3	Forward	CTC AGC CGT ATA TCT TCC CAG
		Reverse	TTG CTT TCT CAG AGG GAT GC
Slc2a1	NM_011400.3	Forward	GAT TGG TTC CTT CTC TGT CGG
		Reverse	CCC AGG ATC AGC ATC TCA AAG
Hk2	NM_013820.3	Forward	TCA AAG AGA ACA AGG GCG AG
		Reverse	AGG AAG CGG ACA TCA CAA TC
Ldha	NM_010699	Forward	GCT CCC CAG AAC AAG ATT ACA G
		Reverse	TCG CCC TTG AGT TTG TCT TC
Gfpt1	NM_013528.3	Forward	CGG CAG TTC TAT ATC AAG GGA G

		Reverse	ACA TCA CGA GGG ACA CAA AC
Gnpnat1	NM_019425.2	Forward	AGT TGT GGA AGA TGT GAC CC
		Reverse	CAG TTT GCC GAG TTG CTT C
Pgm1	NM_025700.2	Forward	TCA AGT GGA TGG GAA ACA GAG
		Reverse	GCC AGC TCT GCA CAA ATA AC
Pgam2	NM_028132	Forward	GGA CAT CCT CAA AGA CCA CTG
		Reverse	AGA ACT GCT TCC CCA CAA AG
Pgk1	NM_008828.2	Forward	AAC CTC CGC TTT CAT GTA GAG
		Reverse	GAC ATC TCC TAG TTT GGA CAG TG
G6pdx	NM_008062.2	Forward	GCC AAC CGT CTA TTC TAC CTG
		Reverse	CAG TTG ATT GGA GCT CTG TAG G
G6pd	NM_000402.3	Forward	AGA ACA TTC ACG AGT CCT GC
		Reverse	GTG GTC GAT GCG GTA GAT C
Pgls	NM_025396.3	Forward	CTT ACC ATC AAT CCT GCC CTA C
		Reverse	GGG AAG AGC GAA CAG GTA TG
Pgd	NM_001081274.1	Forward	GGA GGG AAC AAA GAG GCT TG
		Reverse	CCA TAC TCT ATC CCG TTG TGC
Rpia	NM_009075.2	Forward	AAC ACC CAG AGA TTG ACC TTG
		Reverse	GAA ACG ACT TGC ATA ACC AGC
Rpe	NM_025683.2	Forward	GTA ATG ACC GTA GAA CCT GGG
		Reverse	TCC GCA CAC TTC TGA ACA G
Tkt	NM_009388.5	Forward	TGA AGA GCA AGG ATG ACC AAG
		Reverse	GCT TGA TAG TGA AGG GAT CCA G
Taldo1	NM_011528.4	Forward	GCA TCC TTG ATT GGC ATG TG
		Reverse	ATG GTC TTG TAG CCG AAC TTC
Tktl1	NM_031379.2	Forward	CTT ATT GGT TGT CAC TGC GG
		Reverse	GAG ACG GCA TCA CTT GGA TAG

Tktl2	NM_028927.3	Forward	GGA GTC GGA CAC CTT TCA GG
		Reverse	GCG CTT CTC ATA AAC GGC TG
Pgc-1 alpha	NM_008904.2	Forward	AATCAGACCTGACACAACGC
		Reverse	GCATTCCTCAATTTCACCAA
Pgc-1 beta	NM_133249.2	Forward	CGCTCCAGGAGACTGAATCCAG
		Reverse	CTTGACTACTGTCTGTGAGGC
Pprc1	NM_001081214.1	Forward	AATACCTGGCCGAATGACTC
		Reverse	AGTTGTCACCTTGGACACGA
Atp5g1	NM_001161419.1	Forward	AGTTGGTGTGGCTGGATCA
		Reverse	GCTGCTTGAGAGATGGGTTC
Ndufs8	NM_001271444.1	Forward	TGGCGGCAACGTACAAGTAT
		Reverse	CCTCGGATGAGTTCTGTCCA
Idh3	NM_029573.2	Forward	CCTCCTGCTTAGTGCTGTGA
		Reverse	CGTTGCCTCCCAGATCTTT
Cox5a	NM_007747.2	Forward	GGGTCACACGAGACAGATGA
		Reverse	GGAACCAGATCATAGCCAACA
Cytochrome c	NM_007808.4	Forward	GGAGGCAAGCATAAGACTGG
		Reverse	TCCATCAGGGTATCCTCTCC
Mcad	NM_007382.5	Forward	GAAGGTTGAACTCGCTAGGC
		Reverse	GCTAGCTGATTGGCAATGTC
Ucp2	NM_011671.4	Forward	AAAGGGACCTCTCCCAATGT
		Reverse	GGTCGTCTGTCATGAGGTTG
beta Actin	NM_007393.3	Forward	GACATGGAGAAGATCTGGCA
		Reverse	GGTCTCAAACATGATCTGGGT

mtDNA primers

Cox2		Forward	GCCGACTAAATCAAGCAACA

	Reverse	CAATGGGCATAAAGCTATGG
Cytochrome b	Forward	CATTTATTATCGCGGCCCTA
	Reverse	TGTTGGGTTGTTTGATCCTG
Glucagon gDNA	Forward	CAGGGCCATCTCAGAACC
	Reverse	GCTATTGGAAAGCCTCTTGC
Beta Globin gDNA	Forward	GAAGCGATTCTAGGGAGCAG
	Reverse	GGAGCAGCGATTCTGAGTAGA

Reverse transcriptase PCR primers

Pim1	Forward	GCC CTC CTT TGA AGA AAT CC
	Reverse	GGA CCT GGA GTC TGG AAT GA
Pim2	Forward	ACA TGG TCT GTG GGG ACA TT
	Reverse	TCC TTT GGA GGA GTT GAT GG
Pim3	Forward	AGC AGT GAC CTC TGA CCC CT
	Reverse	TCA AGT ATC CAC CCA GGG CA
beta Actin	Forward	TGA AAC AAC ATA CAA TTC CAT CAT GAA GTG TGA
	Reverse	AGG AGC GAT AAT CTT GAT CTT CAT GGT GCT

SUPPLENMENTAL REFERENCES

1. Cai H, Memarzadeh S, Stoyanova T, Beharry Z, Kraft AS, Witte ON. Collaboration of Kras and Androgen Receptor Signaling Stimulates EZH2 Expression and Tumor-Propagating Cells in Prostate Cancer. *Cancer Res* 2012; **72**: 4672-4681.

2. Beharry Z, Mahajan S, Zemskova M, Lin YW, Tholanikunnel BG, Xia Z *et al*. The Pim protein kinases regulate energy metabolism and cell growth. *Proc Natl Acad Sci USA* 2011; **108**: 528-533.

3. Goldstein, A. S. *et al.* Purification and direct transformation of epithelial progenitor cells from primary human prostate. *Nature Protoc* 2011; **6:** 656-667.

4. Song, J. H. & Kraft, A. S. Pim kinase inhibitors sensitize prostate cancer cells to apoptosis triggered by Bcl-2 family inhibitor ABT-737. *Cancer Res* 2012; **72:** 294-303.

5. Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C *et al*. An integrated clinico-metabolomic model improves prediction of death in sepsis. *Sci Transl Med* 2013; **5**: 195ra95.

6. Weiner J, 3rd, Parida SK, Maertzdorf J, Black GF, Repsilber D, Telaar A *et al*. Biomarkers of inflammation, immunosuppression and stress with active disease are revealed by metabolomic profiling of tuberculosis patients. *PLoS One* 2012; **7**: e40221