Supplemental Experimental Procedures

Generation of conditional SRPK1 knockout mice

Specific restriction fragments containing SRPK1 genomic sequences were isolated from a mouse 129SV/J λ clone, as indicated in Figure S1, and cloned into the pBKSII vector, as previously described. Upon transfection into mouse ES cells at the UCSD mouse core facility, recombinant clones were identified by Southern blotting using PCR-generated 5' probe (5'-GAGCCACCATGTAGGTACTGGGGGAAAAATCGAGGTCTTCTGCAAGAGCAGCCA GTGTTTAACTACAAAGCCACCTCTCCAGCTTCCTTCTAAATTTGTAGCCTGTCTTT TGAAACCTCAGGGAATTACTGTGACCTCCAAAGCACACTCTAAATCAGCTCCTTG GTCTGAATGTCAGCTTCCCCTATAGAACCTCCATTTTGAAATTATTCAGGTAAAAT TCAGCACTTCCTTCCTTAAATGTTGCAATGCTGTTAAAATAGTGTGTGCATGCTTG TCCTTCACCCACTCAGCCCGCCTCTGTAGCCCATCTTGGCCACTATCCTACAAGAA CGTGGATTTTTCTGGACAGTAGGACCTTAATGGGAATCTGCACTTTGCCTGTGGA AGTCAAGATAGGAGATGGTGGCTTCAGATACATGAGGGCTTCTTAGTTCTTCTCA TGGGGAGTTGTGCACACAGTGAAGCACACTCTCTTCTGTTCCTGGCTGTAGCTTCT GTGTCTAGAAAAGTGCTGGCA-3') and 3' probe (5'-GTACATGGGGTGCTTTGCTTGAGTCAGACCCTCAGGAGGAATTTCTG TTTTCATTTGTTCACAAAACAGCAGAATTGTACTCACTGATTATTTTAGAGATAGT AACAACATTAAAGAGACTGGTATTTCTTCCAAATGGGAAGAAAATGAACAGTTGT TATTTTTTTAGATAAAAAGTATTCGATATAGTAAAATAGCTGACTTACATTATTGT TTTAGAGTGTTTTTATTTGATGGGCTTTTCCATTTGATTGACTCCTTTGTAGGGGCA TGTAGCTCAGTATATGTGGGTGTTAGGACAGCTTTCAGGAGTTAGTTTTCTCTGCC

ACGTGGTTCCTGGGGGTCAGACCAAGGTACTGAGGCAGCATCTGTCTTTGCCTGC
TAAACCAGGCTTCTGACCACTGTCATTTTCATTTTTTAGGATGTCTTCTTATACAG
TATGTAGGCCAGGCTGAATTTGAATGCCTGATCTTTCTGTCTTAGGCTCCCCGGGG
CCTAGATTGTAGCTGTATGACACTGGGTGCTCCCTGGGTGGCTGGCTGTGGTTTGC
CTCTTGGTGCTCACACATGCTCACCTACTGACACTGTCAAGTAGCAAGTGCTGCT
GGATTGTGTTTATCGAGGAGACCTTGGCCCAGTTAGGAACTGTGGGAGTTGGACT
CCCGTGGTGCAGCCGGGCGAAGCA-3'). SRPK1 targeted ES clones were injected into C57BL/6J blastocysts and chimeric mice were crossed to C57BL/6J females to obtain germ line-transmitted mice. Three PCR primers [SRPK1-L (5'-ATGTTGGGGTCTGCCAATAA-3'), SRPK1-R (5'-AACTCCAGGCTCTGGTGAGA-3'), 1NeoR (5'-AGCAGCCGATTGTCTGTTGTG-3')] were used for PCR genotyping on both floxed and Cre-deleted alleles. WT allele gives rise to a 375 bp band; the floxed allele generates a 455 bp band; and Cre-deleted allele produces a 550 bp band.

Cell culture and preparation of MEFs

LinX cells were maintained as described (Wang et al., 2001). HeLa cells and HEK293T cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS (Omega Scientific) and 100U of penicillin/streptomycin (Life Technology). Stable cell lines expressing indicated cDNAs were generated by lentiviral transduction in the presence of 8μg/mL polybrene followed by selection with appropriate antibiotics.

Primary mouse embryonic fibroblasts (MEFs) were isolated from C57/BL6 mouse embryos of 14.5- to 15.5-day from crosses between SRPK1 w/f mice by using a published protocol (Serrano et al., 1997). MEFs were genotyped and cultured for 2 passages in DMEM

plus 10% FBS, and penicillin/streptomycin before infection with retrovirus expressing the large T antigen or the Cre recombinase. All the MEF cells used for the experiments were isolated from the same crosses.

Plasmids

Retroviral vectors for expressing the large T antigen (in pBabe-puro vector) and Cre recombinase (in pBabe-hygro vector) were from Dr. Peiqing Sun's laboratory at the Scripps Research Institute. pRL-TK was purchased from Promega. pCMV-myc-SRPK1-WT, pCMV-myc-SRPK1-KD, and pcDNA3-FLAG-SRPK2 were described previously (Zhou et al., 2012). To construct doxyclycline (DOX)-inducible SRPK1 in MEFs, the SRPK1 full-length cDNA was cloned into the pInducer-20 vector (gift of Dr. Stephan Elledge), except that the Neomycin-resistant gene was substituted with the Zeocin-resistant gene.

Retrovirus-mediated gene transduction and RNAi

The retroviral vectors (15μg) for the large T antigen and Cre recombinase were each transfected into the packaging cell line LinX by $CaCl_2$. The viral supernatant was collected 48 h after the transfection and used to infect MEFs. Positively transduced cells were selected with 200μg/ml hygromycin B (for Cre recombinase) or 4μg/ml puromycin (for the large T antigen) for 3 days. The lentivirus system was used to construct DOX-inducible SRPK1 cell lines by infecting MEFs with the supernatant from transfected HEK293T cells according to the Mirus Bio's TransIT protocol.

The siRNA pools against SRPK1 and SRPK2 were purchased from Dharmacon. HeLa and HEK293T cells were transfected with siRNAs using lipofectamine (Invitrogen) at the

final concentration of 1 nM. After transfection with individual siRNAs for 36 hrs, cells were subjected to Western blotting.

Western blotting and co-IP

The sources for primary antibodies used in the study are as follows: SRPK1 and SRPK2 (BD Transduction Laboratories), α-Tubulin and β-Actin (Sigma), Akt-pan, p-Akt-T308, p-Akt-S473, p-Akt-T450, Rictor, p-Rictor, Raptor, p-Raptor, PRAS4, p-PRAS40, GSK3β, p- GSK3β, TSC2, p-TSC2, AMPKα, p- AMPKα, PDK1, p-PDK1, JNK, p-JNK, p38, p-p38, 4E-BP1, p-4E-BP1-Ser65, p-4E-BP1-T37/46, S6K and p-S6K (Cell Signaling), ERK1/2 and p-ERK1/2 (Santa Cruz Biotechnology), mAb104 (ATCC), SRSF2 (GeneTex), SRSF3 (AVIVA Systems Biology), SRSF6 (One World Lab), PHLPP1 and PHLPP2 (Bethyl Laboratories) and HA (Thermo Scientific).

For Western blotting analysis, cell lysates were resolved on SDS-PAGE and transferred to nitrocellulose membrane. The membrane was blocked in buffer TBST plus 5% nonfat milk and then incubated with individual primary antibodies. After extensive rinse with TBST, the blot was incubated with appropriate HRP-conjugated secondary antibody and analyzed by ECL.

For co-IP, 90% confluent cells of were lysed in 1 ml of lysis buffer (10 mM Tris [pH 7.4], 100 mM NaCl, 2.5 mM MgCl2, 0.5% Triton X-100, 1xphosphatase/protease inhibitors), and then incubated with 60 µl of slurry (1:1 ratio) of protein A/G Sepharose beads pre-bound with various antibodies. After incubating overnight at 4°C with rotation, antigen/antibody complex were collected, washed and eluted in 30 µl of 2xSDS loading buffer. The samples were analyzed by Western blotting.

In vitro kinase and phosphatase assays

Myc- or HA-tagged recombinant proteins were expressed and purified from bacteria. The kinase assay was carried out in a 25µl kinase reaction containing 25 mM ATP and 2.5 mCi of [γ-32P]-ATP and incubated for 20 min at 30°C. Reactions were terminated by boiling in 10 µl 2xSDS sample buffer for 5 min. Samples were analyzed by SDS-PAGE and autoradiography. The phosphatase activity in SRPK1 immunoprecipitates was measured using para-nitrophenylphosphate (*p*NPP) as substrate, as described previously (Brognard et al., 2007). Briefly, dephosphorylation of *p*NPP was measured by continuously monitoring the change in absorbance at 405 nM using a Thermo Electron Corp. Genesys 10 UV-visible spectrophotometer. Phosphatase activity was normalized to the amount of SPRK1 in the immunoprecipitates.

Analysis of published expression data

The gene expression profiling data from normal human tissues and cancers were downloaded from the GEO database under accession number GSE7307 and GSE2109 (exp*O* project), respectively, which were generated on Affymetrix U133 plus 2.0 arrays. Raw data from a total of 2835 arrays were processed jointly with Robust Multi-array Average (RMA) approach for background correction and normalization using Bioconductor package affy (Gautier et al., 2004). Each gene expression value was calculated based on the optimal probe set (Li et al., 2011). Data are displayed when tissues or cancers have at least 5 independent samples in individual groups.

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