Supporting Information

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SI Materials and Methods

Generation of Mice Harboring an *Iptk1* Gene Trap Allele. The ES cell line used to produce the transgenic mice was generated with the trapping construct pGT0pfs, containing the intron from the engrailed 2 gene upstream of the gene encoding the βgal/neomycin-resistance fusion protein. C57BL6 mice were obtained from The Jackson Laboratory. PCR-based genotyping of mice was carried out using standard techniques. The primers were 5′-ATTGCACGCAGGTTCTCCGG-3′ and 5′-GGGTCACGACGAGATCCTCG-3′. PCR conditions were 30 cycles at 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min. DNA was prepared from 0.2 cm of clipped tail from 15-day-old mice and purified using the Wizard Genomic DNA Purification Kit (Promega) in accordance with the manufacturer's protocol, using 1 μL of final DNA solution per 50 μL of PCR. Reaction products were electrophoresed on 1% agarose gel and stained with ethidium bromide. To distinguish heterozygotes from homozygotes, PCR was performed using a single sense oligonucleotide 5′-GAGCTGTCTCTCCGTCCTTGTGATTT-3′ and 2 antisense oligonucleotides, 261 bp into the gene trap vector, 5′-CGCCATACAGTCCTCTTCACATCCAT-3′, and 3′ to the gene trap integration site, 5′-AGTACAGCAATGAACAGCCTATCTCTG-3′.

Embryo Collection. Mice were euthanized by carbon dioxide inhalation, and whole embryos or isolated tissue were either frozen immediately in Tissue Tec cryopreservation solution (Sakura Finetek) or fixed overnight in 4% paraformaldehyde in PBS (pH 7.2), and then embedded in paraffin. Then 10-μm cryostat sections were prepared from E15 to P1 for in situ hybridization and immunohistochemistry, and 5-μm sections from the paraffin-embedded embryos were used for immunohistochemistry. For each embryonic stage, sections from at least 2 embryos were analyzed.

Preparation of ITPK1 Antibody and Western Blot Analysis. A recombinant fusion protein between GST and residues 124–311 of human ITPK1 was prepared in *Escherichia coli*, purified by chromatography on a glutathione agarose column, and used to immunize rabbits (Pocono Rabbit Farm and Laboratory). Lysis buffer for brain homogenates contained 20 mM Hepes (pH 7.6), 140 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM EGTA, 1 mM $MgCl_2$, 10 mM benzamidine, 40 μM iodoacetamide, 1 μM pepstatin A, 40 μM leupeptin, 10 μM bestatin, 50 μg/mL of chymostatin, 2 μM calpain inhibitor, 10 μg/mL of antipain, 200 μg/mL of soybean trypsin inhibitor, 1 mM PMSF, and 0.5 mM sodium vanadate. After centrifugation, the clear lysate was removed and frozen in aliquots at -80 °C. For SDS/PAGE, 2.5–6 μg of total protein was applied per lane, transferred to a PVDF membrane (Millipore), and blocked with PBS containing 5% dry milk and 0.1% Tween 20. The membrane was incubated with anti-ITPK1 (1:5,000 dilution) in blocking buffer, followed by incubation with goat-anti-rabbit IgG (HRP) (Pierce). The blot was developed with Supersignal West Pico ECL reagent (Pierce) using standard x-ray film.

Immunostaining, Xgal Staining, and In Situ Hybridization. For immunostaining, the primary antibody was rabbit anti-ITPK1 (1:1,000 dilution), and the secondary antibody was goat anti-rabbit biotinylated IgG (NEF-813, 1:2,000 dilution; NEN Life Science). The avidin-biotin immunoperoxidase system (Vectastain Elite ABC Kit; Vector Laboratories) and diaminobenzidine (Sigma Aldrich) were used to visualize the bound antibody. Slides were then counterstained with 100% hematoxylin. For Xgal staining, frozen sections (10 μm) were prepared by embedding late-gestation mouse embryos or isolated organs from postnatal animals in OCT (Tissue-Tek). Sections of E12.5 mouse embryos were then fixed with 0.2% glutaraldehyde in PBS for 10–20 min; permeabilized with 100 mM potassium phosphate (pH 7.4), 0.02% Nonidet P-40, and 0.01% sodium deoxycholate for 5–15 min; and then incubated in 0.5 mg/mL of Xgal (Promega) with 10 mM $K_3[Fe(CN)_6]$, 10 mM $K_4[Fe(CN)_6]$, 100 mM potassium phosphate (pH 7.4), 0.02% Nonidet P-40, and 0.01% sodium deoxycholate at 30 °C overnight. Xgal-stained sections were counterstained with eosin (52). Whole-mount embryos were postfixed with 1% gluteraldehyde in PBS, embedded in OCT, cryosectioned, and counterstained with eosin (52). For in situ hybridization, sections were incubated in a final volume of 80 μL with 10^6 cpm of $[^{33}P]$-labeled antisense riboprobe prepared using $[^{33}P]$UTP (3,000 Ci/mmol; ICN Biomedicals), T7 polymerase, and 1,400-bp mouse *Itpk1* cDNA cloned in pGEM and linearized with HindIII.

Skeleton Staining. Embryos were harvested at various times, eviscerated, and fixed overnight in 100% ethanol. They were then transferred to acetone for an additional 24 h, skinned, and refixed briefly. After a water rinse, the embryos were stained in 0.2% alizarin red S in 95% ethanol: 0.3% alcian blue 8GX in 70% ethanol: 70% acetic acid: 100% ethanol (1:1:1:17) for 4–6 h at 37 °C, and then at room temperature for 24 h. The embryos were then treated with 1% trypsin in 30% saturated sodium borate solution at 37 °C until the ribs became clear. After removal of any remaining internal organs, skeletons were treated with 1% KOH, rinsed in water, and stored in 20% glycerol.

Scanning Electron Microscopy. Embryos were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and postfixed in 2% OsO_4 in 0.1 M sodium phosphate buffer (pH 7.2). The specimens were then dehydrated with ethanol, transferred to isoamyl acetate, and dried in a critical-point drier using CO_2 as the transitional fluid. The dried specimens were coated with gold and examined with an Akashi ALPHA-30 scanning electron microscope at 30 kV (55).

Fig. S1. Immunoperoxidase staining of wild-type mouse brain showing ITPK1 immunoreactivity in periventricular neurons (*A*) and choroid plexus cells (*B*). (Scale bar = 30 μm.)

Fig. S2. Correlation of Xgal staining and *Itpk1* mRNA expression in a mouse heterozygous for the *Itpk1*–βgal allele. Adjacent cryosections were subjected to Xgal staining (*A* and *C*) or in situ hybridization for *Itpk1* mRNA (*B* and *D*; dark-field microscopy). Shown are sections containing brown adipose tissue (bat), skeletal muscle (sk), and cervical ganglion (cg) cells. (Scale bar = 50 μm.)

Fig. S3. Expression of *Itpk1* in the developing mouse. Mouse embryos containing the *Itpk1*–βgal gene trap allele were subjected to in situ hybridization for *Itpk1* mRNA (A; dark-field microscopy) or Xgal staining (*B–D*). (*A*) At E7.5, *Itpk1* mRNA is expressed in decidua (d) but not in the embryo proper. The amniotic cavity (ac) and extraembryonic coelomic cavity (ecc) are highlighted. The dashed line indicates the orientation of the tissue section shown in the inset. (Scale bar = 50 μm.) (*B*) At E8.5, βgal expression is evident in the paraxial mesoderm (pm) but not in the cranial neural fold (cnf) or anterior intestinal portal (aip). (Scale bar = 50 μm.) (*C* and *D*) At E10.5, βgal expression is seen in the neural tube (nt), trigeminal ganglion (v), and dorsal root ganglia (drg). (Scale bar = 200 μm.)

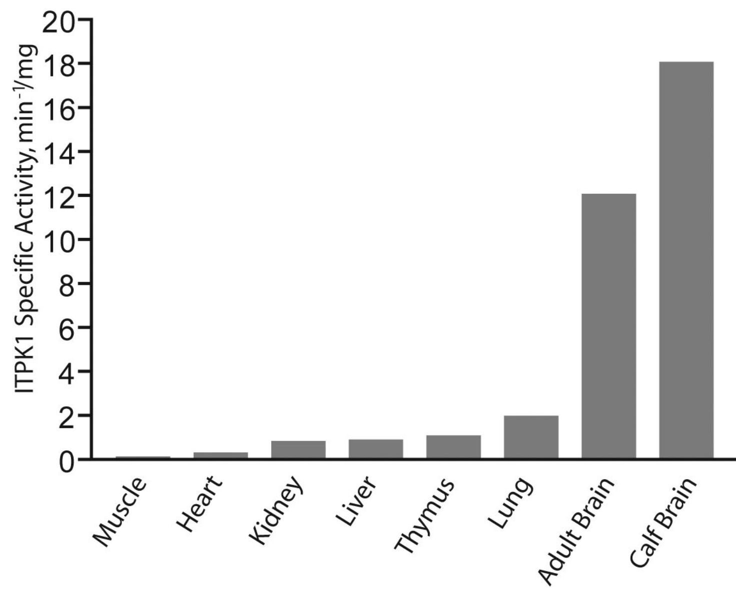

Fig. S4. Enzymatic activity of ITPK1 in bovine tissues. Soluble extracts of bovine tissues were prepared and assayed for phosphorylation of I(1,3,4)P_3 as described previously (14). ITPK1 activity is expressed as a first-order rate constant, k_1, where $[S] = [S]_0e^{-kt}$.

Fig. S5. Growth retardation in mouse embryos homozygous for the *Itpk1*–βgal gene trap allele. Each panel shows a pair of littermates. (Scale bar = 200 μm.)