Supporting Information

Bang et al. 10.1073/pnas.1119751109

SI Materials and Methods

Animals and Housing. IPMK transgenic mice were housed individually in acrylic cages that had a mesh wire bottom. The animal room was maintained on a 12 h:12 h light:dark cycle at 22 ± 2 °C. The animals were given ad libitum access to water and food. Food intake and body weight were measured daily throughout the experiment (Global Diet-2018; Harlan Teklad). All procedures were approved by the Institutional Animal Care and Use Committee of The Johns Hopkins University.

Arc Injections. For Arc administration of GFP-Cre or GFP, mice were anesthetized with an i.p. injection of a mixture of ketamine (100 mg/kg) and dexmedetomidine (0.5 mg/kg) at a volume of 11 mL/kg of body weight. The fur at the top of the head was removed to expose the area to be incised. A hole was trephined at the stereotaxic coordinates above the Arc and the injector was lowered into the Arc (anteroposterior, 1.6 mm; mediolateral, ±0.2 mm; dorsoventral, −5.8 mm). These coordinates were chosen based on preliminary dye injections in a subset of animals and based on previous publications (1). Injections of GFP-Cre or GFP were delivered bilaterally (100 nL/side) through an internal cannula (26-gauge stainless steel; Plastics One) connected to a 0.5-μL microsyringe with polyethylene tubing. The solution was delivered slowly over a 5-min period. After the injection, the surface of the skull was cleaned with saline and the skin was closed with sutures. The animals received a postoperative i.p. injection of atipamezole (2 mg/kg) to reverse the effects of dexmedetomidine. The animals remained on a heating pad until fully awake and then were returned to their home cage.

Plasmids. The cDNAs for human IPMK [National Center for Biotechnology Information (NCBI) Gene ID 253430] and rat IPMK (NCBI Gene ID 171458) were obtained from Open Biosystems. WT and various IPMK cDNA constructs were amplified by PCR and the products were cloned into pCMV-Myc (Clontech), pCMV-GFP (Clontech), or pCMV-GST (2). A mutation of Tyr174 and Tyr110 in rat IPMK with phenylalanine was introduced by using the QuikChange Site-Directed Mutagenesis kit (Stratagene) with oligonucleotides purchased from Invitrogen.

1. Zheng, et al. (2010) A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake. *Am J Physiol Regul Integr Comp Physiol* 298:R720−R728.

2. Adams J, et al. (2004) Intrasteric control of AMPK via the gamma1 subunit AMP allosteric regulatory site. *Protein Sci* 13:155−165.

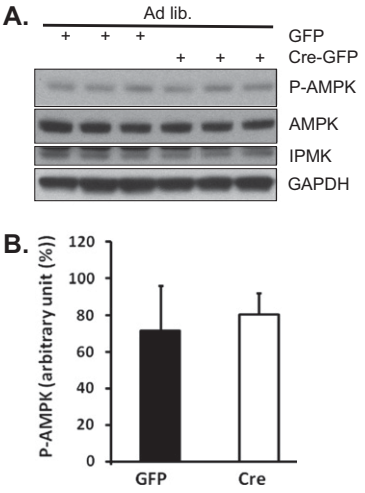

Fig. S1. Effects of refeeding on IPMK gene expression in dorsal hypothalamus. (*A*) *IPMK*$^{1ox/1ox}$ mice were injected with adenovirus expressing either GFP or Cre recombinase in ventral area. Mice were fed ad libitum, and the ventral hypothalamus was isolated and Western blotted for P-AMPK, AMPK, IPMK, and GAPDH. (*B*) Relative quantifications of P-AMPKα expression levels are shown in GFP (black bars) or GFP-Cre (open bars) injected mice. Values are corrected for corresponding total AMPK antibody (Student *t* test; $*P < 0.05$).

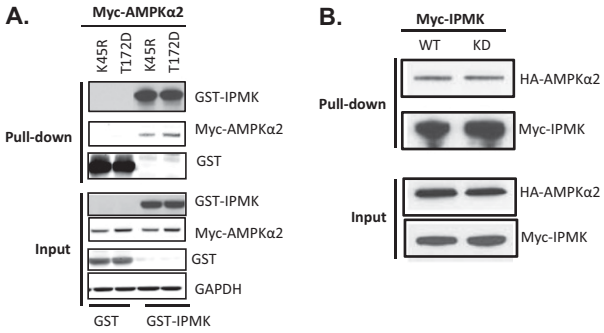

Fig. S2. Catalytic status of either AMPKα2 or IPMK is not required for IPMK-AMPK interaction. (*A*) GST-IPMK was cotransfected in HEK2 93T cells with myc-AMPKα2-T172D (constitutively active) or myc-AMPKα2-K45R (catalytically dead), and GST pull-down was performed. The precipitated proteins and the input proteins were detected by immunoblotting with antibodies to GST or myc. (*B*) HA-AMPKα2 and WT or Kinase-dead mutant (S/A and K/A) of Myc-IPMK were cotransfected into GT1-7 cells as indicated. HA-AMPKα2 was immunoprecipitated and coimmunoprecipitates of Myc-IPMK determined by Western blot.

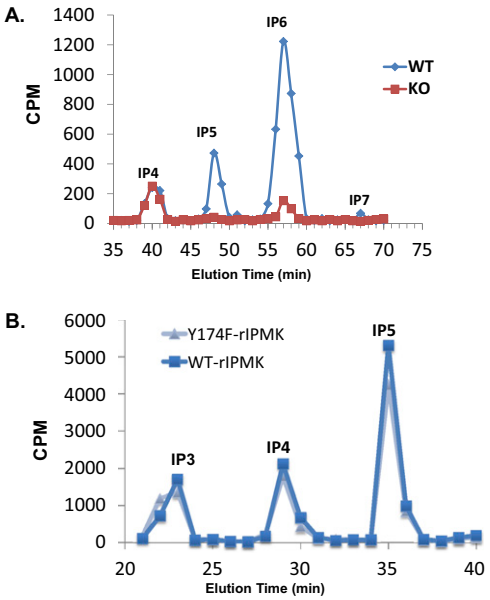

Fig. S3. Comparison of higher-order inositol phosphate profiles of WT and IPMK$^{-/-}$ MEFs (*A*) and overexpressed WT or Y174F mutant of myc-IPMK in 2 93Tcells (*B*). Equal numbers of each cell type were labeled with [^{3}H]myo-inositol. After extraction, inositol phosphates were resolved by HPLC and measured by scintillation counting.