Supporting Information

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SI Methods

Generation of PDK1-MerCre Mice. Mice harboring a *Pdk1*^{flox} allele were previously described (1, 2). Mice expressing a tamoxifen-inducible Cre recombinase protein fused to two mutant estrogen-receptor ligand-binding domains (MerCreMer) under the control of the *α-myosin heavy chain* (*MHC*) promoter were previously described (3). We crossed *Pdk1*$^{flox/flox}$ mice with *αMHC-MerCreMer* mice, and then bred the resulting *Pdk1*$^{flox/+}$/*MerCreMer*$^{+}$ offspring with *Pdk1*$^{flox/flox}$ mice to generate *Pdk1*$^{flox/flox}$/*MerCreMer*$^{+}$ mice (PDK1-MerCre). For induction of Cre-mediated recombination, tamoxifen (Sigma) was i.p. administered to mice once a day for 5 successive days at a dosage of 20 mg/kg/day (3). Bcl2-Tg mice and PIK-Tg mice were kindly gifted by Dr. Michael D. Schneider (Imperial College, London, U.K.) (4) and Dr. Howard A. Rockman (Duke University Medical Center, Durham, NC) (5). All of the experimental protocols were approved by the Institutional Animal Care and Use Committee of Chiba University.

Animal Models. Myocardial infarction was produced by ligation of left anterior descending artery of anesthetized male C57BL/6 mice at 10 weeks of age, as previously described (6). Doxorubicin (20 mg/kg) was administered by i.p. injection to 10-week-old male C57BL/6 mice, as previously described (7). These mice were killed 2 weeks after operation of myocardial infarction or doxorubicin injection.

Measurement of cAMP. We determined cAMP concentration in homogenates of hearts using a cAMP EIA system according to the manufacturer's protocol (GE Healthcare Bio-Sciences).

Antibodies. The following antibodies were used: PDK1, phosphorylated-Akt, Akt, phosphorylated-GSK3*β*, GSK3*β*, phosphorylated-mTOR (Ser-2448), mTOR, phosphorylated-p70S6K, p70S6K, phosphorylated-CaMKII (Thr-286), cleaved PARP (Cell Signaling Technology), phosphorylated-PLN (Ser-16), PLN, SGK1 (Upstate), HSP90, CaMKIIδ (Santa Cruz Biotechnology), Rab4 (BD Biosciences), and actin (Sigma).

Phosphodiesterase Assay. We determined phosphodiesterase (PDE) activity in immunoprecipitates using an IMAP FP Phosphodiesterase Evaluation Kit (Molecular Devices) according to the manufacturer's protocol. The selective PDE3B inhibitor cilostamide (Calbiochem) was dissolved in DMSO and diluted into the reaction mixture at the concentration of IC_{50} (70 nM).

1. Sakaue H, et al. (2003) Requirement for 3-phosphoinositide-kependent dinase-1 (PDK-1) in insulin-induced glucose uptake in immortalized brown adipocytes. *J Biol Chem* 278:38870–38874.
2. Inoue H, et al. (2006) Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* 3:267–275.
3. Sohal DS, et al. (2001) Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circ Res* 89:20–25.
4. Imahashi K, Schneider MD, Steenbergen C, Murphy E (2004) Transgenic expression of Bcl-2 modulates energy metabolism, prevents cytosolic acidification during ischemia, and reduces ischemia/reperfusion injury. *Circ Res* 95:734–741.
5. Perrino C, et al. (2005) Restoration of beta-adrenergic receptor signaling and contractile function in heart failure by disruption of the betaARK1/phosphoinositide 3-kinase complex. *Circulation* 111:2579–2587.
6. Harada M, et al. (2005) G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. *Nat Med* 11:305–311.
7. Toko H, et al. (2002) Angiotensin II type 1a receptor mediates doxorubicin-induced cardiomyopathy. *Hypertens Res* 25:597–603.

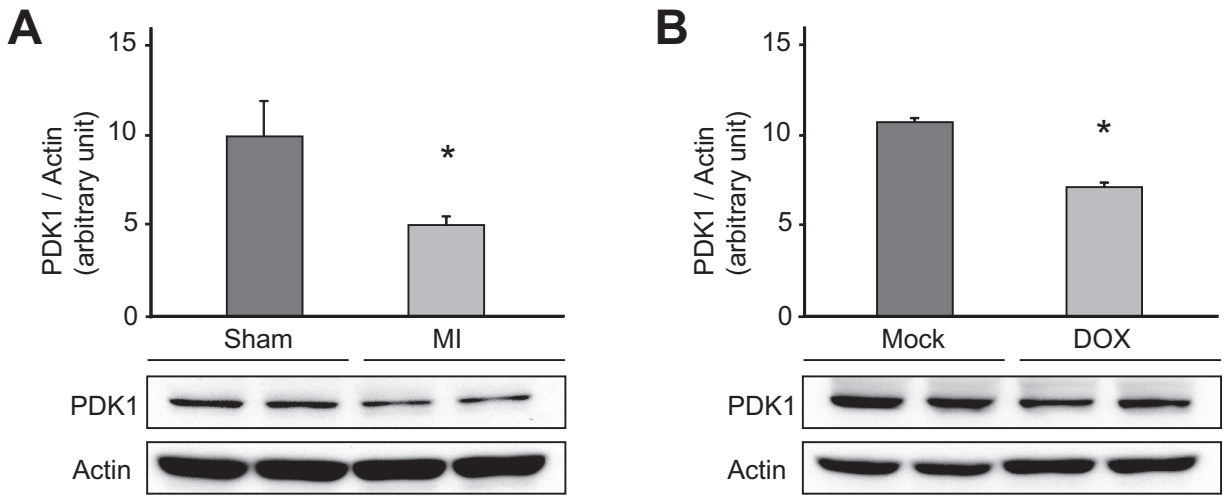

Fig. S1. Expression levels of PDK1 in failing hearts. Immunoblot analysis of PDK1 expression in murine hearts of postmyocardial infarction cardiomyopahty (*A*) and doxorubicin-induced cardiomyopahty (*B*). The intensity of each band was quantified by densitometric analysis and corrected for the amount of actin protein as an internal control. Data are expressed as mean ± SEM of 6 independent experiments. *, $P < 0.05$ versus control group. MI, myocardial infarction. DOX, doxorubicin.

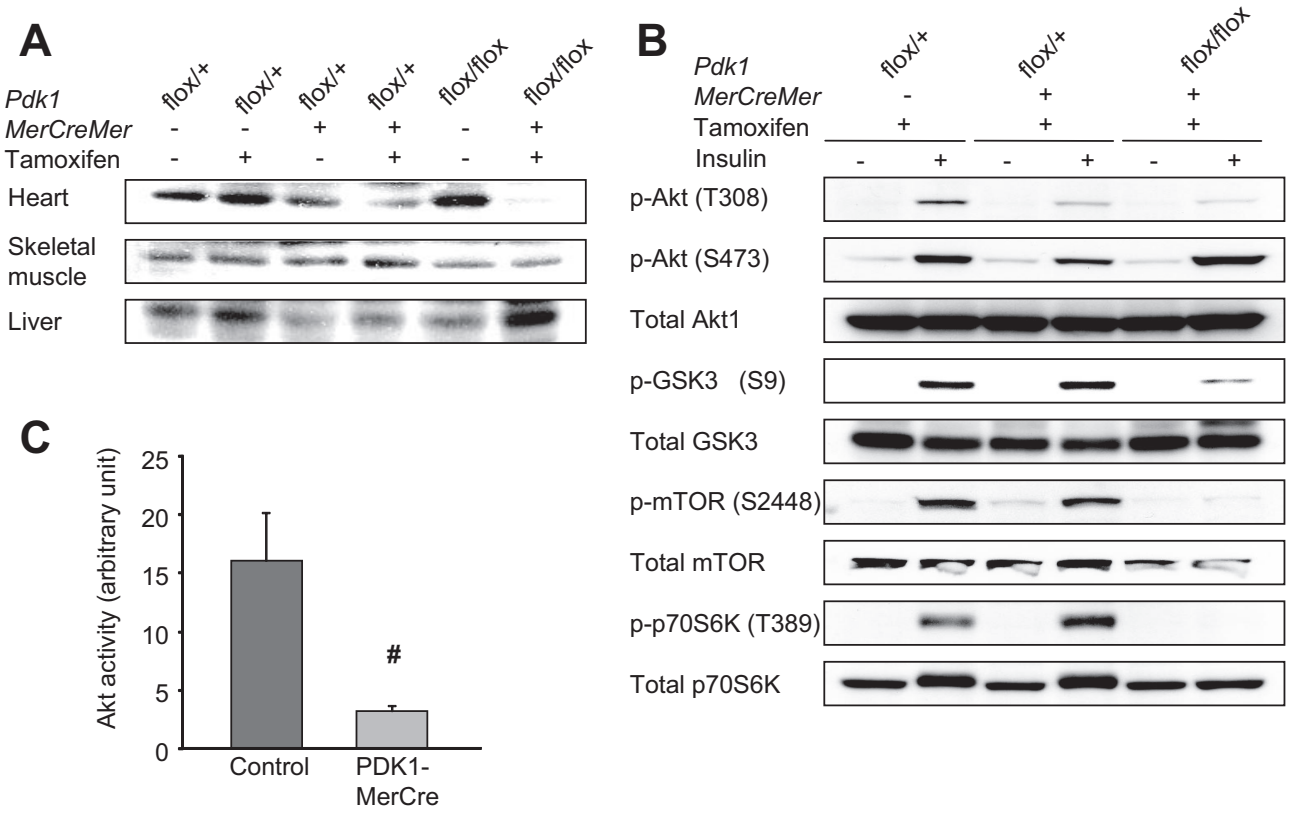

Fig. S2. Generation of PDK1-MerCre mice. (*A*) Immunoblot analysis of PDK1 expression in heart, skeletal muscle, and liver of mice 1 week after tamoxifen treatment. (*B*) Immunoblot analysis of insulin-induced phosphorylations of Akt at Thr-308 or Ser-473, GSK3β at Ser-9, mTOR at Ser-2448, and p70S6K at Thr-389 in the hearts of PDK1-MerCre mice. (*C*) Kinase assay for Akt activity. Values represent the mean ± SEM of data from 5 mice in each group. #, $P < 0.01$ versus control group.

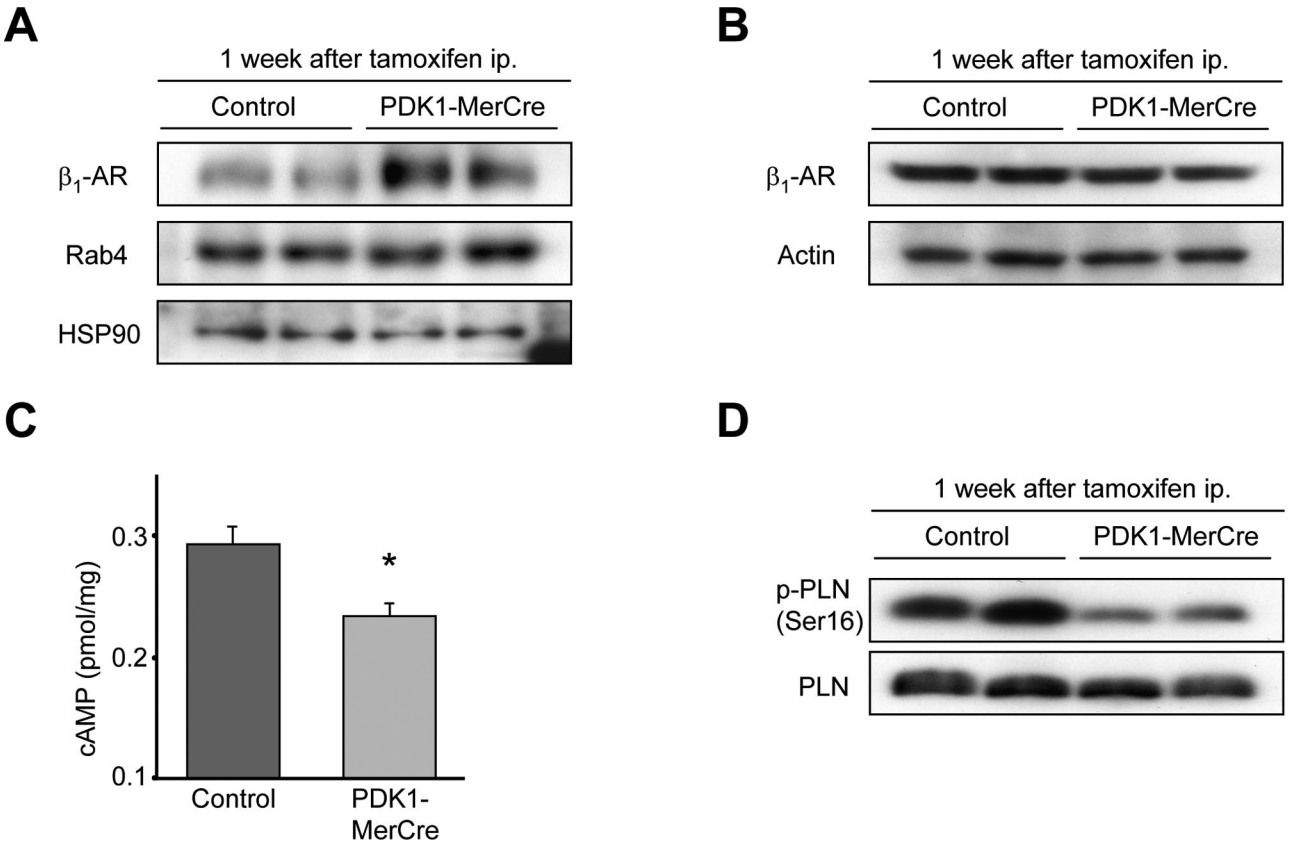

Fig. S3. β-AR signaling in PDK1-MerCre mice. (*A*) Immunoblot analysis of β_1-AR in cytosol fraction of the hearts. Rab4 is a small GTP-binding molecule that is associated with early endosomes and recycling vesicles. Rab4 was detected in cytosol fraction. HSP90 was used as an internal control for the amount of cytosol fraction. (*B*) Immunoblot analysis of β_1-AR in total cell lysate of the hearts. Actin was used as an internal control. (*C*) Cardiac cAMP concentration of PDK1-MerCre mice. Values represent the mean ± SEM of data from control mice (n = 4) and PDK1-MerCre mice (n = 4). *, $P < 0.05$ versus control group. (*D*) Immunoblot analysis of phosphorylated-PLN at Ser-16 in the hearts of PDK1-MerCre mice.

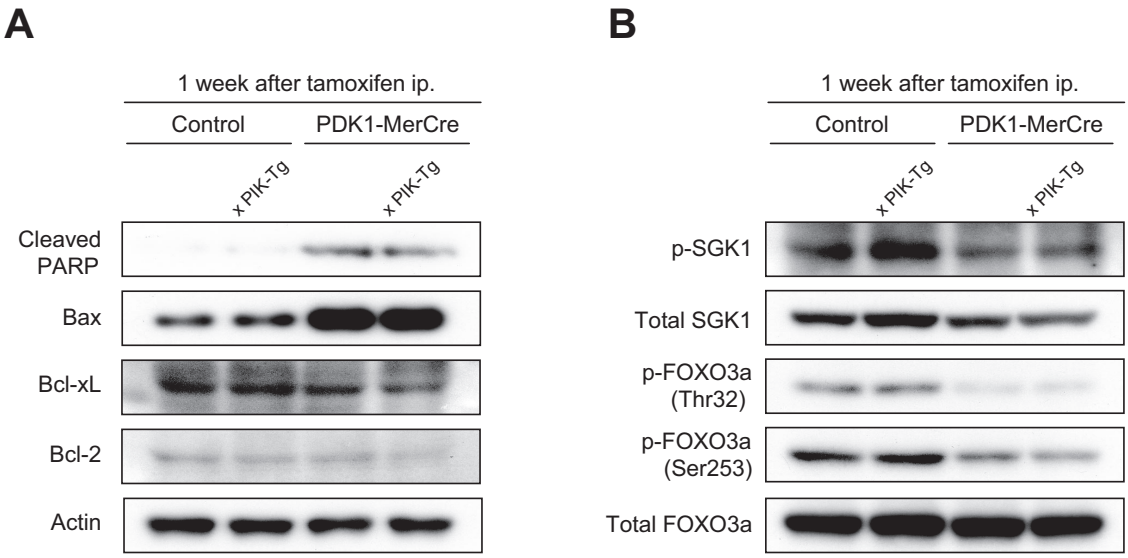

Fig. S4. Apoptotic signaling in PDK1-MerCre x PIK-Tg mice. (*A*) Immunoblot analysis of cleaved poly(ADP-ribose) polymerase (PARP) and Bcl-2 family proteins in the hearts. Actin was used as an internal control. (*B*) Immunoblot analysis of phosphorylated-SGK1 at Ser-78, total SGK1, phosphorylated-FOXO3a at Thr-32 or at Ser-253, and total FOXO3a in the hearts.

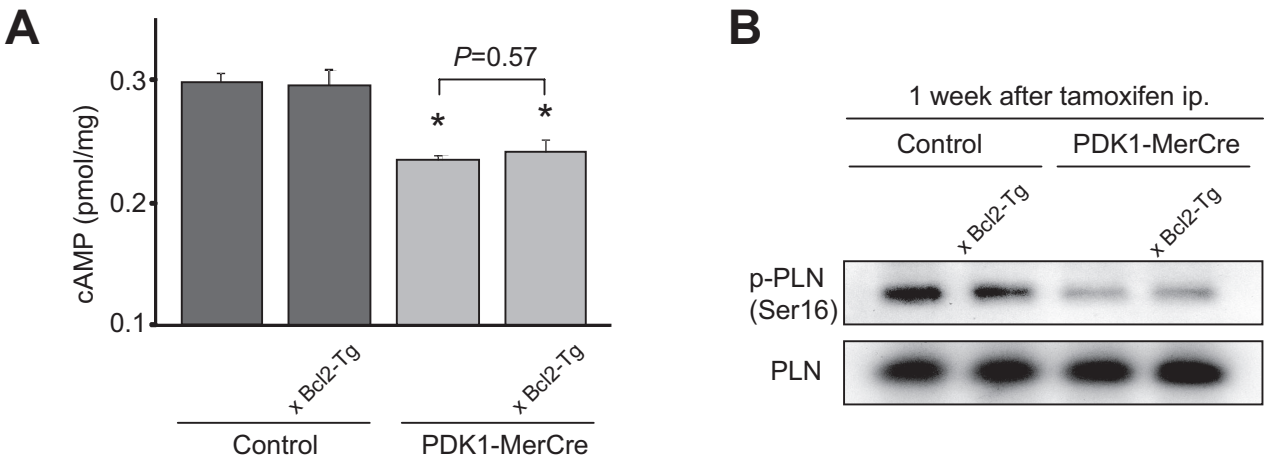

Fig. S5. β-AR signaling in PDK1-MerCre × Bcl2-Tg mice. (*A*) Cardiac cAMP concentration of PDK1-MerCre mice. Values represent the mean ± SEM of data from control mice (n = 4), control × Bcl2-Tg mice (n = 4), PDK1-MerCre mice (n = 4), and PDK1-MerCre × Bcl2-Tg mice (n = 4). *, $P < 0.05$ versus control group. (*B*) Immunoblot analysis of phosphorylated-PLN at Ser-16 in the hearts of control mice, control × Bcl2-Tg mice, PDK1-MerCre mice, and PDK1-MerCre × Bcl2-Tg mice.

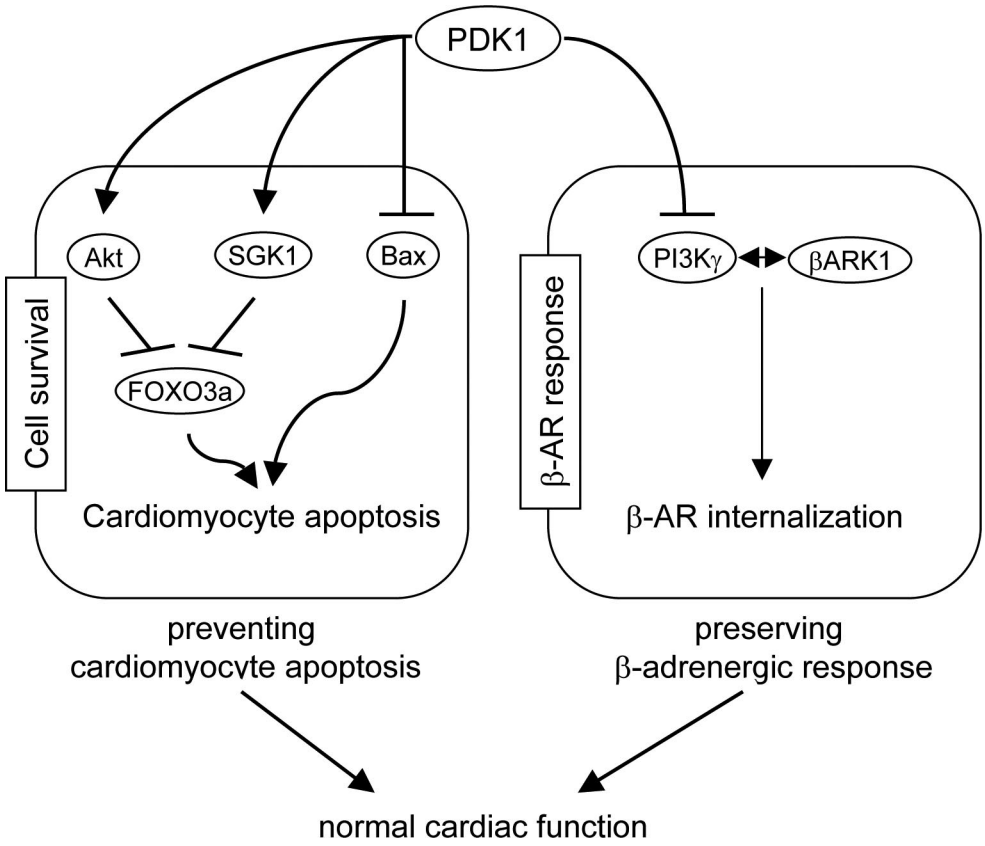

Fig. S6. Schematic summary of the homeostatic effects of PDK1 in the heart. PDK1 promotes survival of cardiomyocytes through activation of Akt and SGK1 and subsequent inactivation of FOXO3a, and through prevention of Bax up-regulation. PDK1 is also involved in the negative feedback regulation of PI3-Kγ. PDK1 preserves β-adrenergic response through prevention of robust β-AR internalization mediated by βARK1/PI3-Kγ complex.

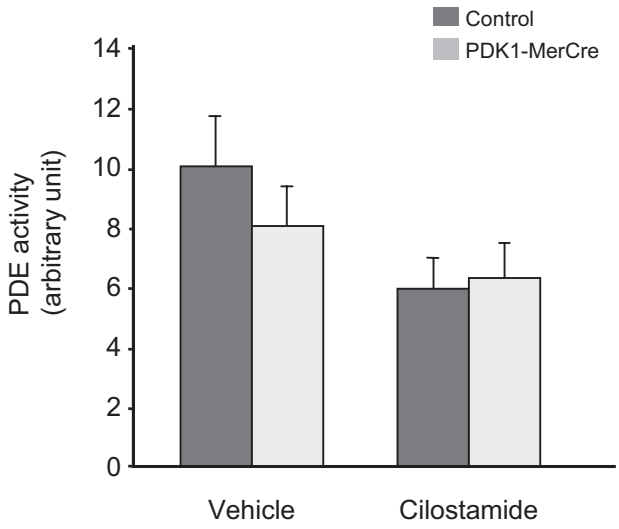

Fig. S7. PI3-Kγ-associated PDE activity in PDK1-MerCre mice. PDE activity immunoprecipitated with antibody to p110γ was indistinguishable between PDK1-MerCre and control hearts ($P = 0.32$). In addition, the PDE activity in immunoprecipitates from PDK1-MerCre and control hearts showed no significant difference in the presence of cilostamide ($P = 0.77$). These results indicate that PI3-Kγ-associated PDE3B activity is not enhanced in PDK1-MerCre hearts. Values represent the mean $\pm$ SEM of data from 4 mice in each group. PDE activity of control mice was adjusted to 10 arbitrary units.

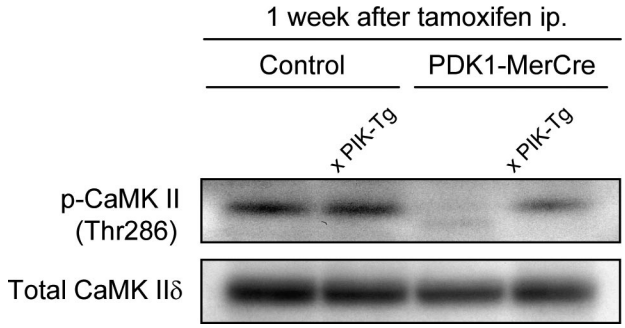

Fig. S8. CaMKII signaling in PDK1-MerCre × PIK-Tg mice. Immunoblot analysis of phosphorylated CaMKII at Thr-286 and total CaMKIIδ, in the hearts of control mice, control × PIK-Tg mice, PDK1-MerCre mice, and PDK1-MerCre × PIK-Tg mice.