Catalucci et al., http://www.jcb.org/cgi/content/full/jcb.200805063/DC1

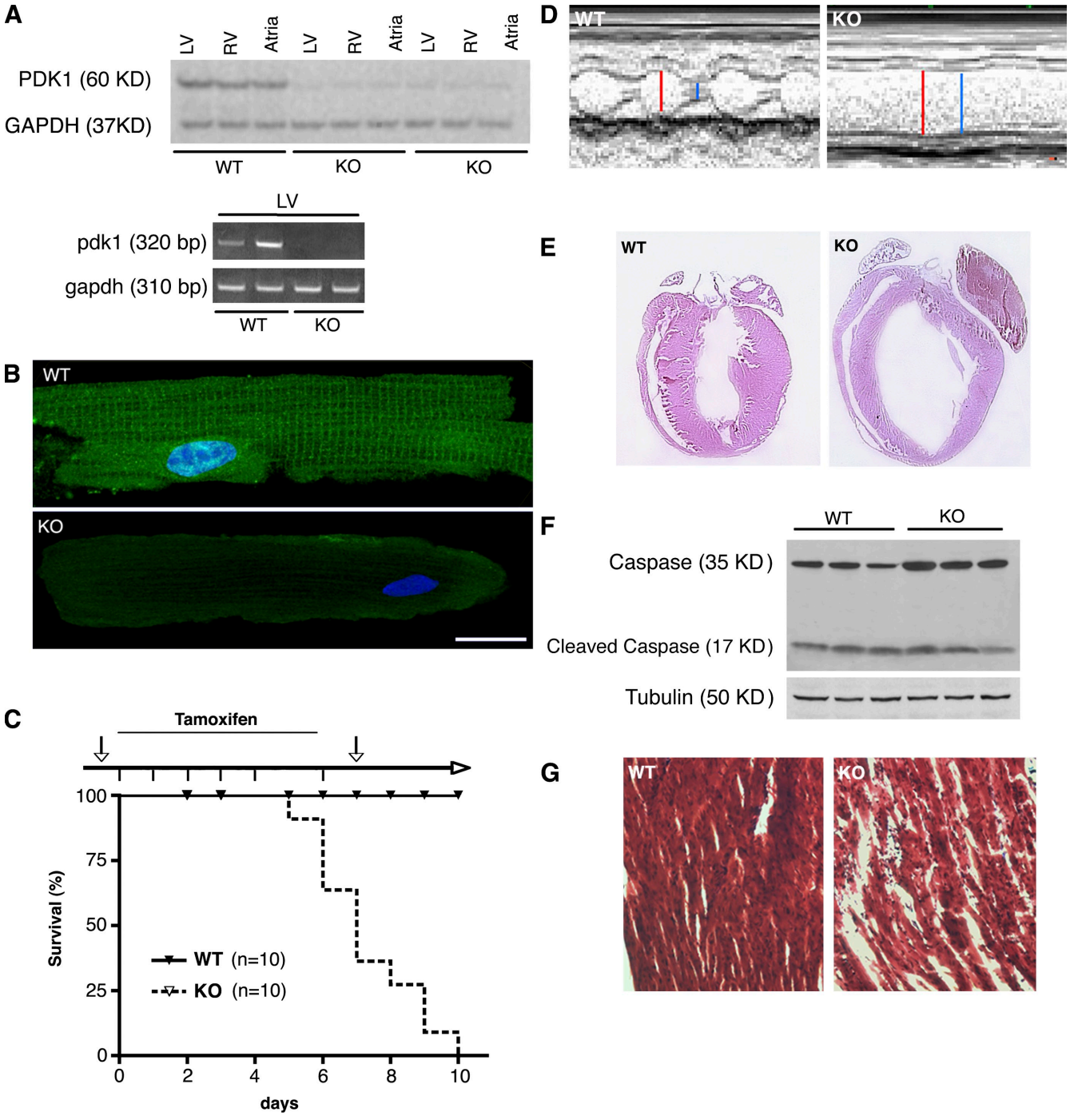

Figure S1. **Characterization of mice lacking PDK1 expression.** (A) PDK1 protein and RNA levels assessed by Western blot (top) and RT-PCR (bottom) analyses of atria and left (LV) and right (RV) ventricular cardiomyocytes from WT and KO mice. Protein and RNA loading were normalized to tubulin and GAPDH levels, respectively. (B) Immunofluorescence staining of cardiomyocytes isolated from WT and KO hearts labeled with antibody against PDK1 (green) and counterstained with Hoechst nuclear stain (blue). (C) Survival curve for mice lacking PDK1 (KO) in the heart. Mortality begins 5 d after tamoxifen injection and reaches 100% by day 10 after the beginning of treatment. Time points of tamoxifen injections (hash marks) and echocardiography analysis (arrows) are shown (n = 10). (D) Echocardiographic (M mode) assessment of left ventricular size and function. Left ventricular diastolic internal dimensions (red lines) and left ventricular systolic internal dimensions (blue lines) were increased in KO mice. Heart rates were 486 bpm and 511 bpm in the WT and KO hearts, respectively. (E) Hematoxylin- and eosin-stained paraffin sections show severe dilatation and thinning of KO hearts. (F) Western blot analysis for caspase 3 activation in WT and KO heart homogenates. Basal apoptotic activation as a consequence of tamoxifen treatment was also observed in WT (Zartman, J.K., N.K. Foreman, A.M. Donson, and J.M. Fleitz. 2004. *J. Neurooncol.* 67:3–7). The amounts of loaded protein were verified with tubulin antibodies. (G) Representative Masson's trichrome staining of tissue sections from WT and KO hearts. Bar, 15 µm.

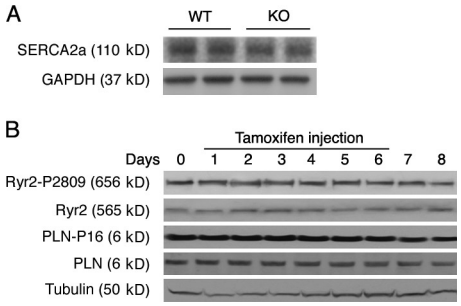

Figure S2. **Regulation of Ca^{2+} handling proteins by Akt.** (A) Western blot analysis of ventricular homogenates from WT and KO mice. (B) Western blot analysis of ventricular homogenates along a time course of tamoxifen inductions (day 1–6 treatment is indicated by the line) using various antibodies. A representative experiment is shown (n = 3).

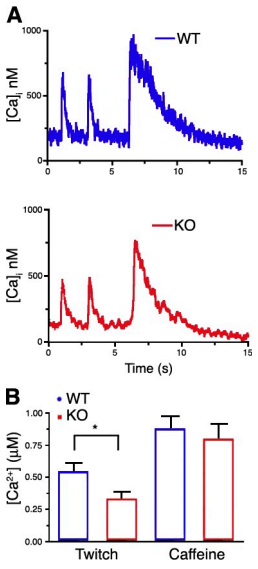

Figure S3. **Altered intracellular Ca handling in PDK1 KO cardiomyocytes.** (A) Representative Ca^{2+} traces are shown for WT (top) and KO (bottom) cardiomyocytes. (B) Reduced averaged twitch Ca^{2+} transient amplitude is shown in KO compared with WT cardiomyocytes (left; unpaired t test; *, $P < 0.05$). No difference was found in total SR Ca^{2+} analysis (right). WT and KO results are shown in blue and red, respectively. Error bars show SEM.

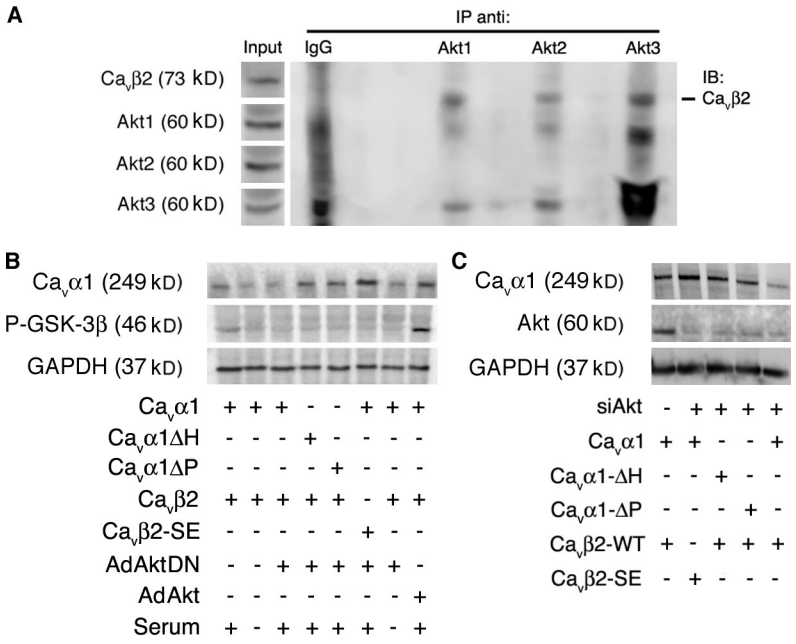

Figure S4. **Ca$_v$β2 interacts with Akt isoforms, and Akt affects Ca$_v$α1 protein stability.** (A) Immunoprecipitated Akt isoforms from WT cardiac extracts from mice treated or not treated with 1 mU/g insulin assayed for Ca$_v$β2. The input protein in each coimmunoprecipitation is shown. IB, immunoblot; IP, immunoprecipitation. (B) Ca$_v$α1-, Ca$_v$α1-ΔP–, or Ca$_v$α1-ΔH–cotransfected 293T cells with either Ca$_v$β2 or Ca$_v$β2-SE were infected with the indicated active (AdAkt) or DN (AdAktDN) Akt-expressing adenoviral vectors. Cells were serum starved overnight as indicated. The expression of Ca$_v$α1 and phosphorylation of glycogen synthase kinase (GSK) in lysates were monitored by Western blot analysis. (C) Ca$_v$α1-, Ca$_v$α1-ΔP–, or Ca$_v$α1-ΔH–cotransfected 293T cells with either Ca$_v$β2 or Ca$_v$β2-SE were treated with siAkt-expressing vector as indicated. 3 d after transfection, cells were lysated for protein extraction. Protein loading was normalized to GAPDH levels. Representative experiments are shown (n = 3).

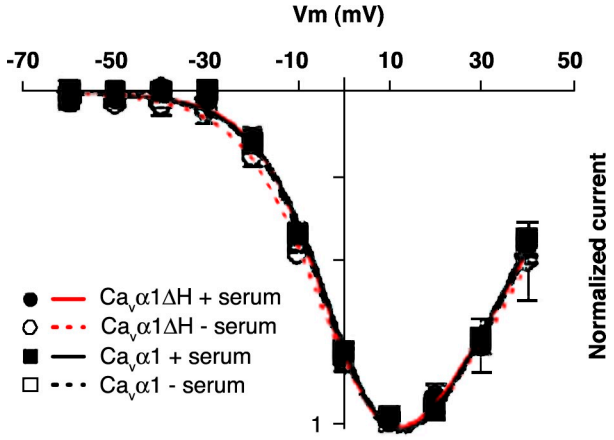

Figure S5. **Serum deprivation and PEST-H deletion does not modify steady-state activation parameters.** Ca^{2+} currents recorded in cotransfected tsA-201 cells with Ca$_v$β2-WT and either Ca$_v$α1-WT or Ca$_v$α1-ΔH and cultivated for 36 h in the presence or absence of 10% fetal bovine serum. I-V curves are normalized to the maximal current. n > 35 in each condition (ANOVA). Error bars show SEM.

Table S1. **Echocardiography analysis of WT and KO mice at day 7 after initiation of tamoxifen injections**

Parameter	Basal (*n* = 10)	KO (*n* = 10)
Heart rate (bpm)	506 ± 5	492 ± 9
Body weight (g)	21 ± 4	21 ± 4
LVIDd/body weight	0.16 ± 0.02	0.20 ± 0.03[a]
IVSd (mm)	0.57 ± 0.02	0.51 ± 0.02[b]
LVIDd (mm)	3.35 ± 0.33	4.24 ± 0.19[b]
LVPWd (mm)	0.59 ± 0.06	0.52 ± 0.01
IVSs (mm)	0.96 ± 0.09	0.67 ± 0.08[b]
LVIDs (mm)	1.80 ± 0.37	3.66 ± 0.28[c]
LVPWs (mm)	1.16 ± 0.09	0.84 ± 0.07[b]
Fractional shortening (%)	46.5 ± 7.02	13.79 ± 5.39[b]
EDD/PWD	5.73 ± 0.60	8.16 ± 0.25[c]
VCF (circ/s)	8.85 ± 1.40	3.19 ± 1.19[b]
LVM (d) (mg)	55.06 ± 12.86	73.89 ± 7.74[a]
LVPWd/LVIDd	0.18 ± 0.02	0.12 ± 0.02[a]
Heart weight/body weight	0.0054 ± 0.0010	0.0067 ± 0.0011[a]

Values are expressed as means ± SD. bpm, beat per minute; LVIDd, left ventricular internal end-diastolic diameter; LVIDs, left ventricular internal end-systolic diameter; IVSd, interventricular septum thickness in diastole; IVSs, interventricular septum thickness in systole; LVPWd, left ventricle posterior wall thickness in diastole; LVPWs, left ventricle posterior wall thickness in systole; EDD, end-diastolic diameter; PWD, posterior wall in diastole; VCF, velocity of circumferential fiber shortening calculated as fractional shortening divided by ejection time multiplied by the square root of the RR interval; LVM, left ventricular mass.
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.