Supplementary information

Supplementary methods:

Immunofluorescence:

Immunofluorescence was performed as described previously (11). Primary antibodies were as follows: an anti-ENH1 monoclonal antibody (Abnova), anti-α1C polyclonal (Alomone labs). The fluorescence of cells was observed using a confocal LSM5 microscope (Carl Zeiss).

Cloning of the C-terminal domain of α1C.

The C-terminal domain of α1C was subcloned from the complete coding sequence of the rabbit cardiac dihydropyridine-sensitive calcium channel (kindly provided by Professor Birnbaumer (National Institutes of Health, Research Triangle Park, North Carolina, USA). To create a construct encoding a C-terminal His fusion protein (1509 to 2170,amino acid counting), we designed two primers: 5'- TAC AAG CTT CCA TGG ACAACT TTG ACT AC CT -3' and 5'- GCT CTA GAC AGG CTG CTG ACG CCG GCC CT-3' and use them for PCR amplification with Pfx polymerase (Invitrogen). The amplicon was digested with HindIII and XbaI (underlined) and cloned into a pcDNA4 vector (Invitrogen). The construct was sequenced.

In-vitro phosphorylation assay.

In-vitro phosphorylation of the C-terminal domain of α1C was performed as described previously (11, 15). Briefly, the C-terminal domain of α1C was immunoprecipitation from Hek cells extract. For the phosphorylation assay the beads were mixed with 25 μl of the reaction mixture containing 20 mM Tris, pH 7.5, 10 mM $MgCl_2$, 20 μM ATP, 20 KBq [γ-^{32}P] ATP (about 220 TBq/mmol), and 10 ng recombinant constitutive active PKD1(Calbiochem), and incubated at 30 ^{o}C for 20 min. After the reaction was terminated by addition of an SDS-sample buffer, the beads were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and subsequent autoradiography using a Phosphor Imaging System Cyclone (Packard).

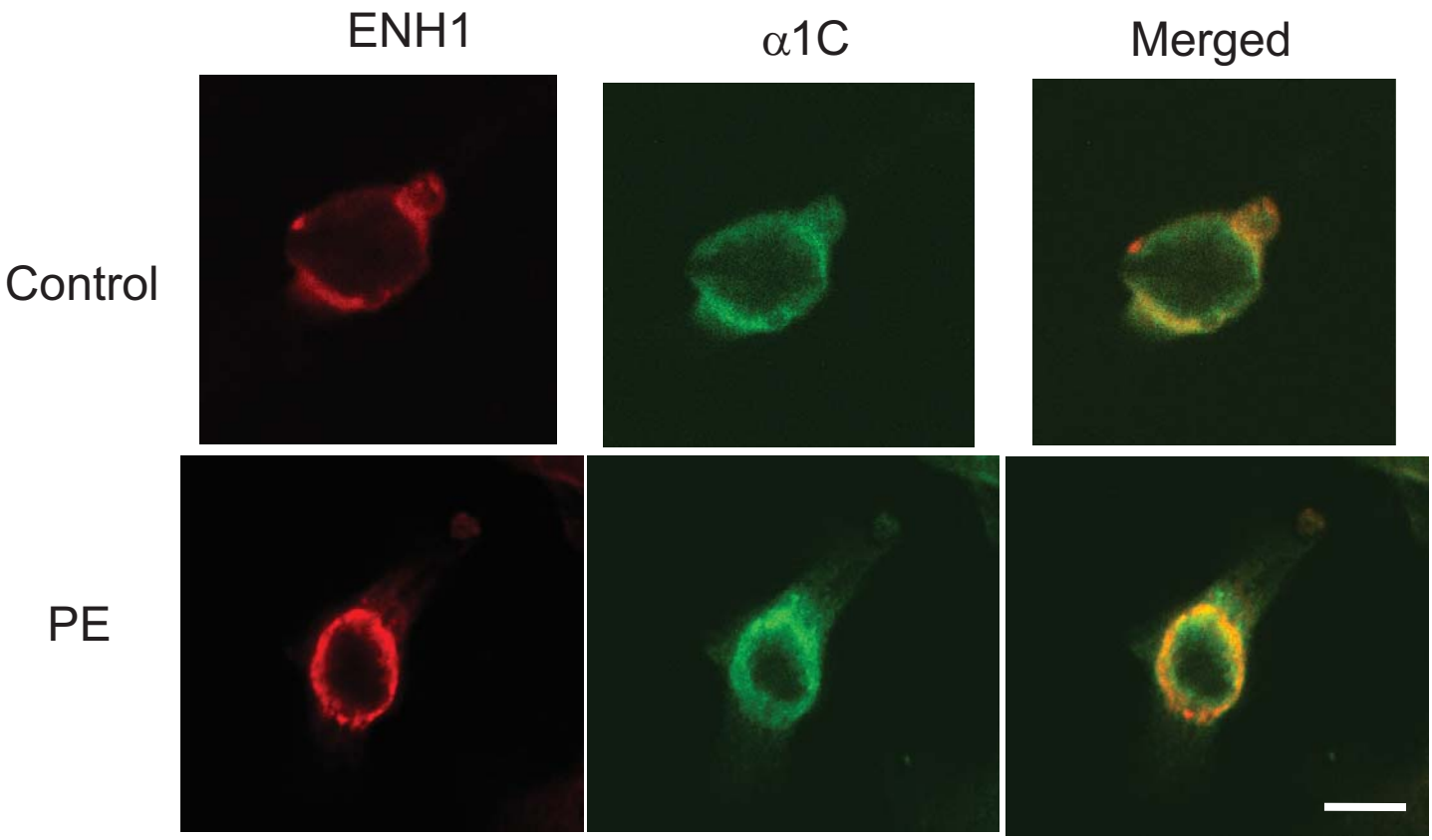
Supplementary figures legends

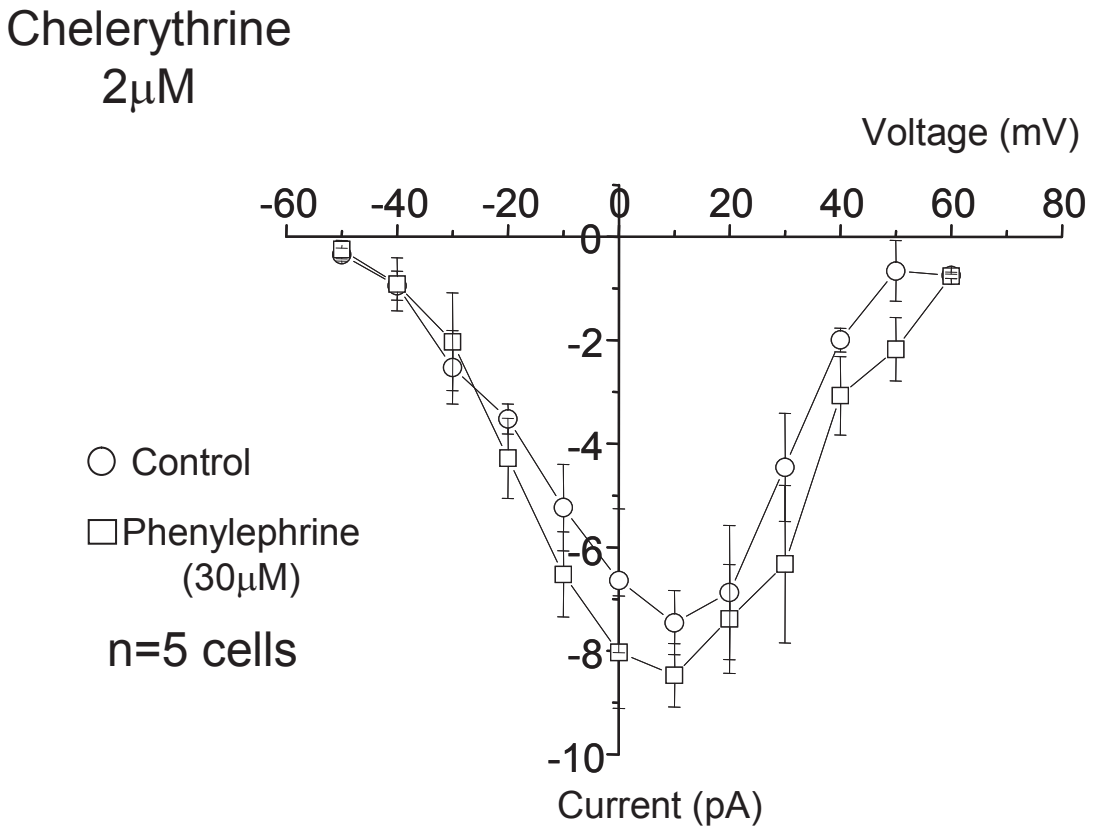
Figure S1*: Colocalisation of ENH1 and α1C in neonatal rat cardiomyocytes.*
Images are immunofluorescence of control and PE-stimulated neonatal rat cardiomyocytes. Cells were co-stained for L-type voltage-gated Ca^{2+} channel using a polyclonal anti-α1C and for ENH1 using a monoclonal anti-ENH1. Images were merged to show co-localization. Scale bare represents 10 μm.

Figure S2: *Inhibition of the PE-induce increase of L-type calcium currents with protein kinase C inhibitors.*
L-type calcium currents were measured in neonatal rat cardiomyocytes by the patch-clamp. Mean current density-voltage relationship of L-type calcium current measured

before and after the addition of 20 μM PE in presence of PKC specific inhibitors, 2 μM Chelerythrine (upper graph) or 2 μM GF109203X (lower graph).

Figures S3: *PKD1 phosphorylates in-vitro the C-terminal domain of the α1C subunit of the L-type voltage-gated* Ca^{2+} *channel.* In-vitro phosphorylation assay was performed using the immunoprecipitated myc-tagged C-terminal domain of the α1C subunit using an anti–myc specific antibody. Autoradiography shows the phosphorylation level of the C-terminal domain by recombinant constitutive active PKD1 (Active-PKD1, left) and wild-type non active PKD1 (Inactive-PKD1, right) were used for in-vitro phosphorylation. Immunoblots show the immunoprecipitated myc-tagged C-terminal domain of the α1C to confirm the expression. In-vitro phosphorylation assay was performed 3 times independently.


ENH1
α1C
Merged
Control
PE


Chelerythrine
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Voltage (mV)
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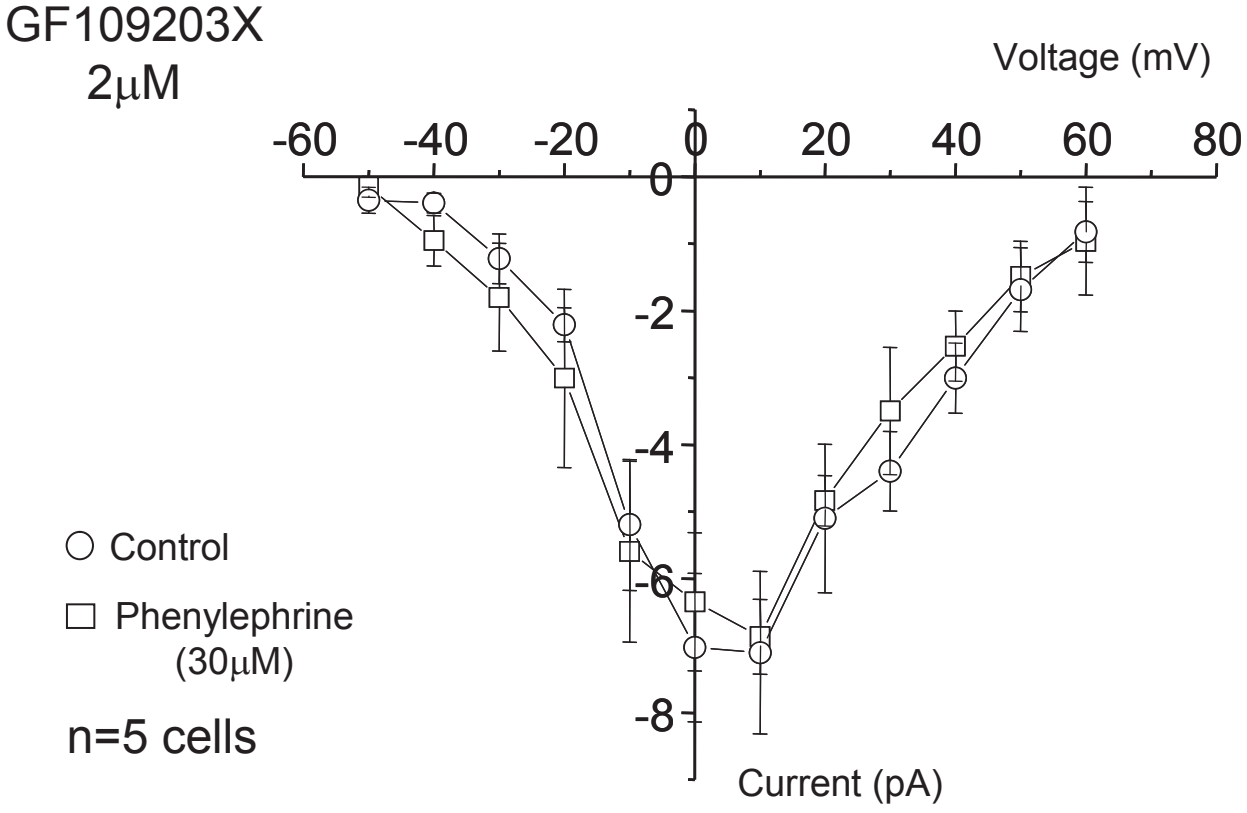
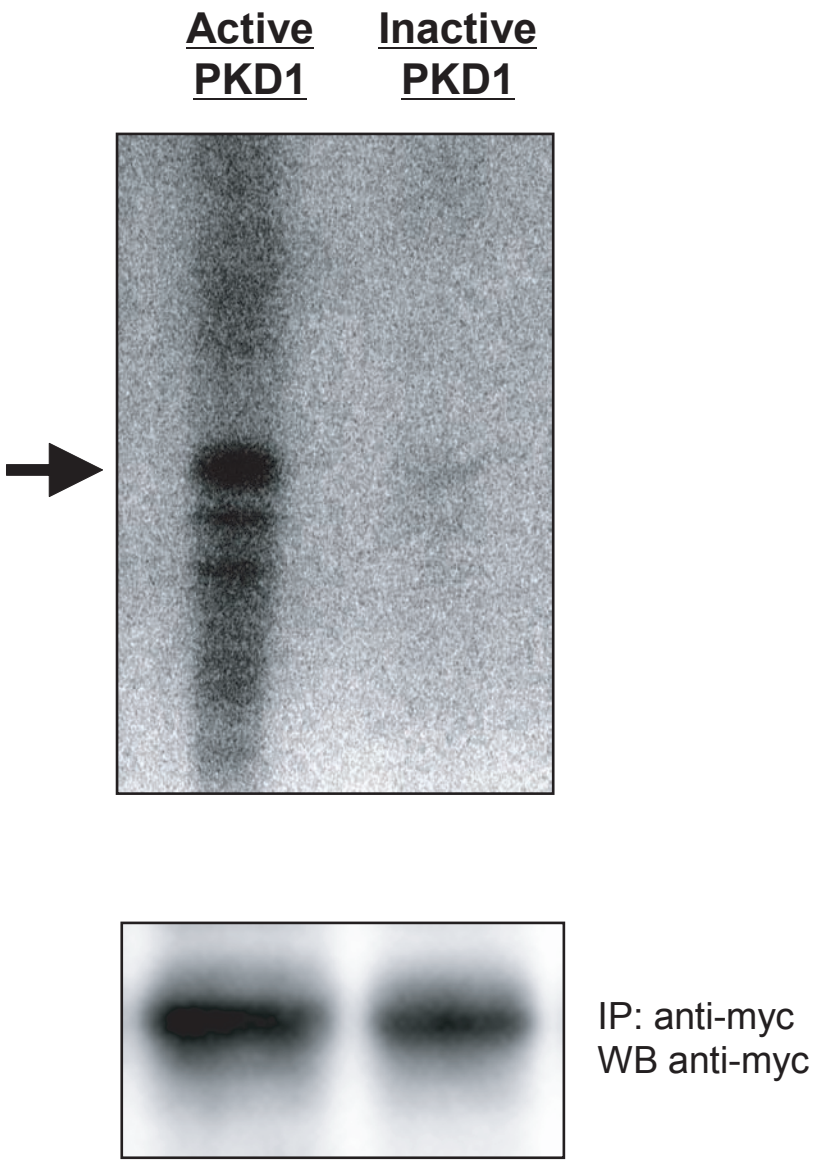
-40
-20
0
20
40
60
80
0
-2
-4
-6
-8
-10
Current (pA)
Control
Phenylephrine
(30μM)
n=5 cells


GF109203X
2μM
Voltage (mV)
-60
-40
-20
0
20
40
60
80
0
-2
-4
-6
-8
Current (pA)
Control
Phenylephrine
(30μM)
n=5 cells


Active PKD1
Inactive PKD1
IP: anti-myc
WB anti-myc