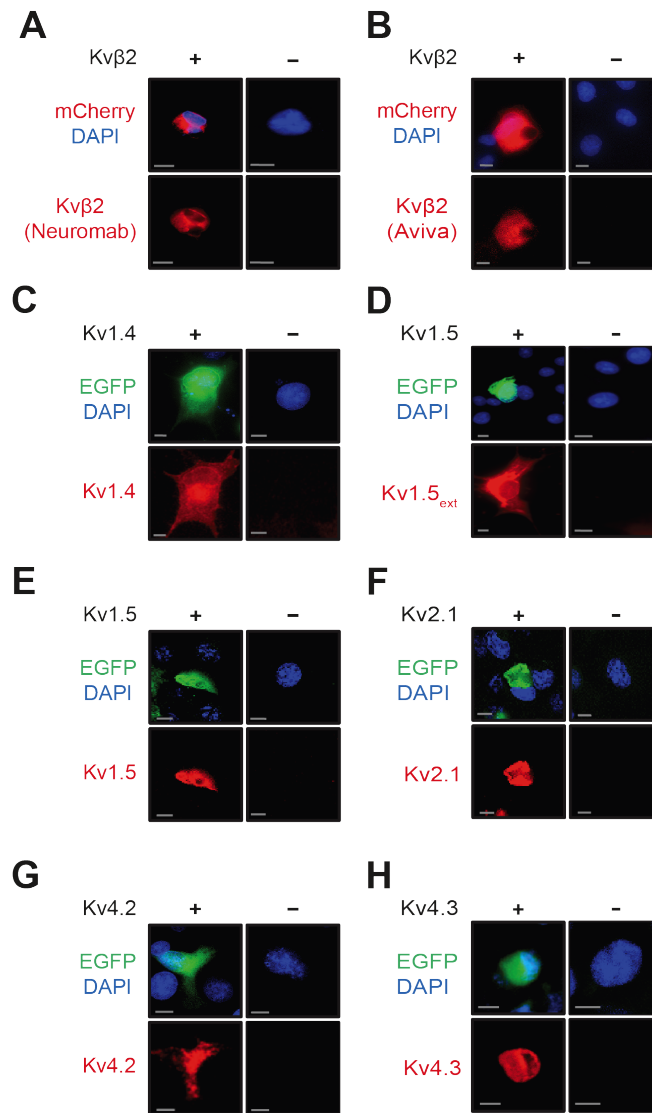**Figure S1: Protein recognition by anti-Kv primary antibodies used in immunofluorescence and PLA experiments.** Widefield fluorescence images of COS-7 cells expressing (+) Kvβ2 (**A,B**; *Kcnab2*; NM_001252656.1), Kv1.4 (**C**; *Kcna4*; NM_0211275.4), Kv1.5 (**D,E**; *Kcna5*; NM_145983.2), Kv2.1 (**F**; *Kcnb1*; NM_008420.4), Kv4.2 (**G**; *Kcnd2*; NM_019697.4), and Kv4.3 (**H**; *Kcnd3*; NM_019931.1) labeled using primary antibodies as indicated and described in Methods. Images of untransfected (−) COS-7 cells that were negative for mCherry or EGFP

reporters and were labeled for respective Kv proteins using the identical procedure are also shown for each. Scale bars represent 10 μm.
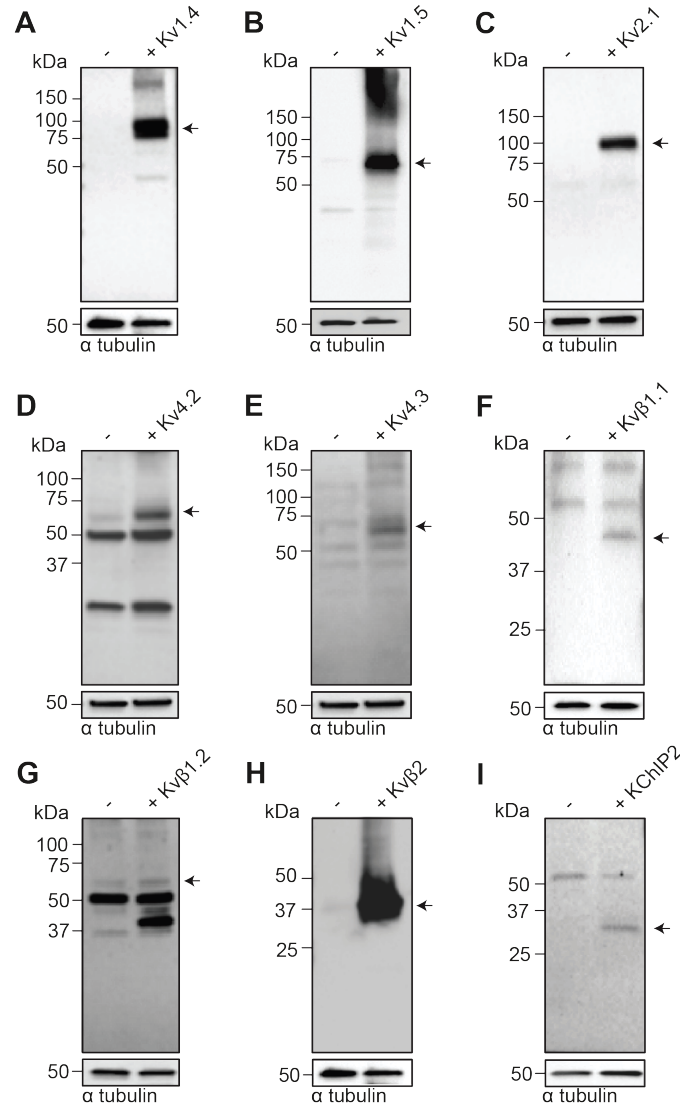
Figure S2: Immunoreactivity of anti-Kv antibodies used in Western blot and co-immunoprecipitation experiments. Western blots showing immunoreactive bands for Kv1.4 (A), Kv1.5 (B), Kv2.1 (C), Kv4.2 (D), Kv4.3 (E), Kvβ1.1 (F), Kvβ1.2 (G), Kvβ2 (H), and KChIP2 (I) in untransfected COS-7 cells (-) and COS-7 cells transfected with expression vectors for target genes (*Kcna4*, NM_0211275.4; *Kcna5*, NM_145983.2; *Kcnb1*, NM_008420.4; *Kcnd2*, NM_019697.4; *Kcnd3*, NM_019931.1; *Kcnab1.1*, NM_010597.4; *Kcnab1.2*, AF131935; *Kcnab2*, NM_001252656.1; KChIP2, NM_145703.2). Arrows depict bands at expected molecular weight for respective protein of interest.
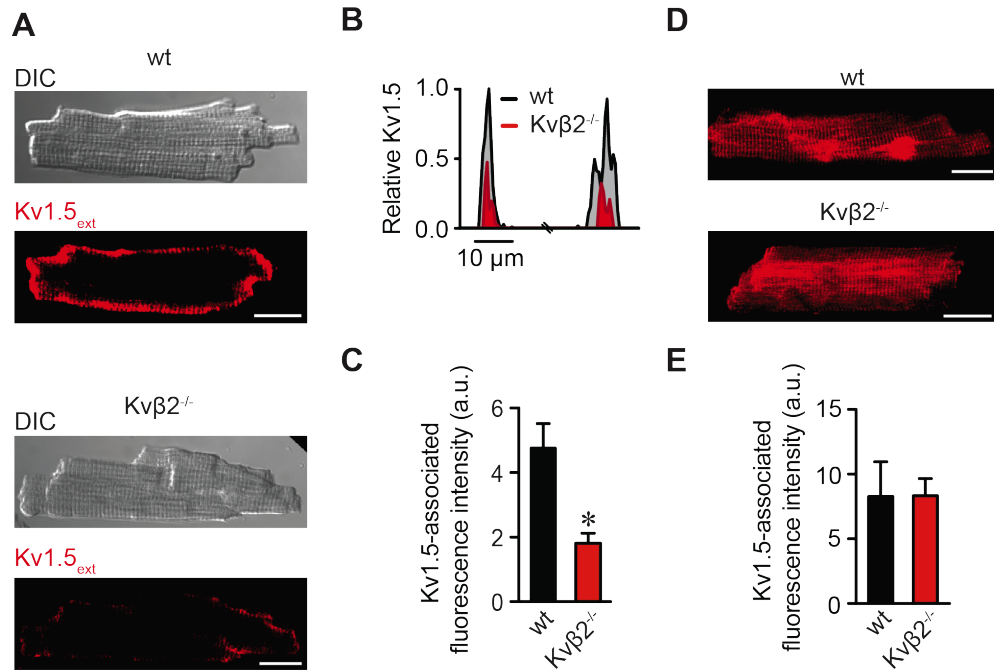
Figure S3: Reduced sarcolemmal abundance of Kv1.5 in cardiac myocytes from Kvβ2$^{-/-}$ mice. (**A**) DIC and confocal images showing membrane-associated Kv1.5 (Kv1.5$_{ext}$, red) in isolated non-permeabilized ventricular myocytes from wt and Kvβ2$^{-/-}$ animals. Scale bars = 20 µm. (**B,C**) Line intensity plots showing relative Kv1.5$_{ext}$-associated fluorescence levels for myocytes shown in panel *A (B)*, and bar plot showing membrane-associated Kv1.5-associated fluorescence intensity (C; arbitrary units) in myocytes from wt and Kvβ2$^{-/-}$ animals (n = 9 cells from 3 animals for each). (**D,E**) Confocal images showing total Kv1.5 (D), and bar plot summarizing mean Kv1.5-associated fluorescence intensities in isolated permeabilized ventricular myocytes from wt and Kvβ2$^{-/-}$ animals (n = 7-8 cells from 3 mice each).

Table S1: Kv current and action potential properties in cardiac myocytes from wild type and Kvβ2$^{-/-}$ animals.

	WT (n = 18)	**Kvβ2$^{-/-}$** (n = 15)
Cell capacitance (pF)	103.5 ± 5.3	93.5 ± 4.4
I_{to}		
pA/pF	27.6 ± 3.5	15.8 ± 2.0*
τ **(msec)**	59.3 ± 1.5	63.8 ± 2.7
$I_{K,slow1}$		
pA/pF	15.6 ± 1.6	12.3 ± 2.3*
τ **(msec)**	418.6 ± 36.2	467.3 ± 27.3
$I_{K,slow2}$		
pA/pF	20.6 ± 1.5	16.3 ± 2.1*
τ **(msec)**	2474.8 ± 179.1	2923 ± 274.7
I_{ss}		
pA/pF	8.7 ± 1.4	8.1 ± 1.0
RMP (mV)	-82.8 ± 1.2	-84.5 ± 0.6
dV/dT$_{max}$ (mV/msec)	153.1 ± 11.0	142.0 ± 9.3
AP amplitude (mV)	113.6 ± 1.8	112.7 ± 1.6
APD$_{20}$ (msec)	1.87 ± 0.06	2.17 ± 0.08*
APD$_{50}$ (msec)	3.77 ± 0.21	4.68 ± 0.34*
APD$_{90}$ (msec)	16.22 ± 1.54	24.31 ± 2.52*

Data are mean ± SEM. *P<0.05.

Table S2: Comparison of electrocardiographic parameters in wild type and Kvβ2$^{-/-}$ mice.

Measurement	Wild type (N = 6)	Kvβ2$^{-/-}$ (N = 10)
RR interval (msec)	134.5 ± 3.5	140.1 ± 2.0
Heart rate (bpm)	448 ± 11	429 ± 6
P duration (msec)	8.5 ± 0.5	11.4 ± 0.7*
PR interval (msec)	43.5 ± 1.5	39.3 ± 2.2
QRS interval (msec)	8.4 ± 0.3	11.7 ± 0.5*
QT interval (msec)	21.7 ± 0.3	26.0 ± 0.6*
QTc (msec)	59.3 ± 1.2	69.5 ± 1.5*
JT interval (msec)	13.3 ± 0.4	14.3 ± 0.5

Data are mean ± SEM. *$P<0.05$.

Table S3: Comparison of echocardiographic parameters in wild type and Kvβ2$^{-/-}$ mice.

Measurement	Wild type (N = 10)	Kvβ2$^{-/-}$ (N = 11)
Age (wks)	21.9 ± 0.1	22.1 ± 0.2
Weight (g)	28.8 ± 1.2	27.5 ± 0.7
HR (BPM)	467 ± 14	463 ± 10
Endocardial values		
EDV (µl)	37.2 ± 2.6	36.0 ± 2.2
ESV (µl)	11.0 ± 1.5	11.8 ± 1.4
SV (µl)	26.2 ± 1.5	24.2 ± 1.2
EF (%; M)	80 ± 3	72 ± 2*
EF (%; B)	71 ± 2	68 ± 2
FS (%)	49 ± 3	41 ± 2*
CO (ml/min)	12 ± 0.7	11 ± 0.5
E/A	1.59 ± 0.08	1.53 ± 0.09
Chamber diameter		
LVIDd (mm)	3.5 ± 0.1	3.5 ± 0.1
LVIDs (mm)	1.8 ± 0.1	2.0 ± 0.2
Wall Thickness		
LVPWd (mm)	0.8 ± 0.1	0.8 ± 0.1
LVPWs (mm)	1.3 ± 0.1	1.1 ± 0.1*
LVAWd (mm)	0.8 ± 0.1	0.7 ± 0.1
LVAWs (mm)	1.1 ± 0.1	1.0 ± 0.1
RWT	0.46 ± 0.02	0.43 ± 0.01

Data are mean ± SEM. *P<0.05. Abbreviations: HR, heart rate; BPM, beats per minute; EDV, end diastolic volume; ESV, end systolic volume; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; LVIDd, left ventricular inner diameter at diastole; LVIDs, left ventricular inner diameter at systole; LVPWd, left ventricular posterior wall at diastole; LVPWs, left ventricular posterior wall at systole; LVAWd, left ventricular anterior wall at diastole; LVAWs, left ventricular anterior wall at systole; RWT, relative wall thickness.