**SUPPLEMENTARY MATERIAL**

# Deletion in mice of X-linked, Brugada syndrome- and atrial fibrillation-associated *Kcne5* augments ventricular Kv currents and predisposes to ventricular arrhythmias

Jens-Peter David[1=], Ulrike Lisewski[2=], Shawn M. Crump[3], Thomas A. Jepps[1], Elke Bocksteins[4†], Nicola Wilck[2¥], Janine Lossie[2Ŧ], Torsten K. Roepke[2#], Nicole Schmitt[1#], Geoffrey W. Abbott[3#*]

[1]Danish National Research Foundation Centre for Cardiac Arrhythmia and Dept. of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

[2]Charité - Universitätsmedizin Berlin- Medizinische Klinik und Poliklinik m. S. Kardiologie und Angiologie, CCM and Experimental and Clinical Research Center (ECRC), Lindenberger Weg 80, 13125 Berlin, Germany

[3]Bioelectricity Laboratory, Dept. Physiology and Biophysics, University of California, Irvine, CA, U.S.A.

[4]Laboratory for Molecular Biophysics, Physiology and Pharmacology, Department for Biomedical Sciences, University of Antwerp, Antwerp, Belgium

[=]equally contributing authors; [#]equally contributing authors

[†]present address: Quality by Design NV, 2610 Wilrijk, Belgium

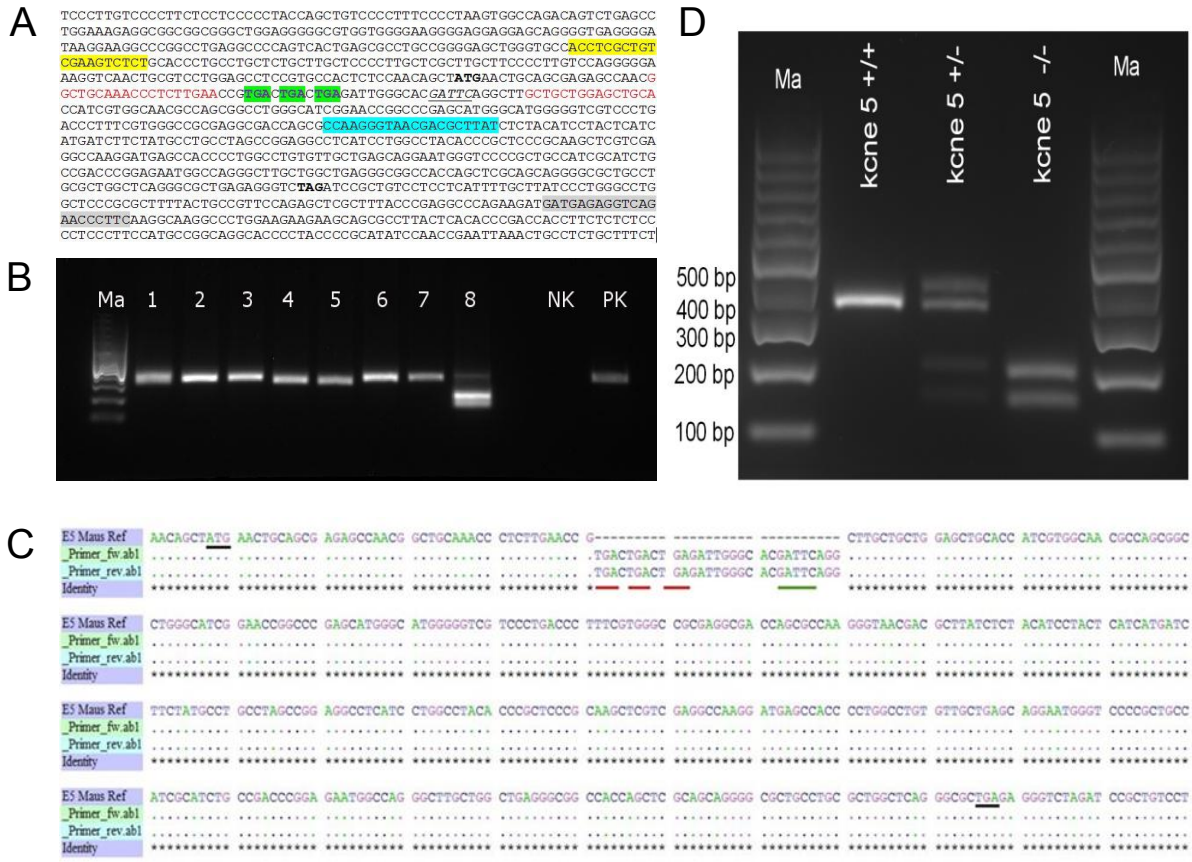
[Ŧ]present address: Omeicos Therapeutics, Robert-Rössle-Straße 10, 13125 Berlin, Germany

[¥] present address: Medizinische Klinik mit Schwerpunkt Nephrologie und Internistische Intensivmedizin Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany and Experimental and Clinical Research Center (ECRC), Lindenberger Weg 80, 13125 Berlin, Germany

*CORRESPONDING AUTHOR:

Prof. Geoffrey W. Abbott, Bioelectricity Laboratory, Med Sci D337, Department of Physiology and Biophysics, School of Medicine, University of California, Irvine, CA 92697, U.S.A.

Phone: 1-949-824-3269; E-mail: abbottg@uci.edu




**Supplementary Figure S1.** Schematic of a successful homology directed repair HDR of the designed ZFNs and donor vector for *Kcne5*. **A.** Schematic for the donor vector sequence designed to introduce three stop-codons (TGA) for every reading frame (green/purple) as well as an exogenous TfiI restriction site (underlined) into the mouse genome *in vivo*. Also shown are endogenous start (ATG) and stop (TAG) condon (bold/black) of *Kcne5*. ZFN binding site (red) and primers for PCR (yellow/blue) and sequencing (yellow/grey). **B.** Detection of introduced TfiI site in founder animals. Founder mice were genotyped by TfiI digestion of the 320 bp PCR product amplified from ear genomic DNA (1% agarose gel). The PCR product from wildtype mice, lacking the TfiI restriction site, could not be digested (lane 1-7), whereas the PCR product from sucessful ZFN/donor vector treatment could be digested by TfiI (lane 8) into two distinct fragments. (Ma – marker, PK – positive control, NK – negative control). **C.** Sequence analysis of the *Kcne5* mutant allele of one founder with correct TfiI restriction bands. The target region of the mouse Kcne5 gene was PCR-amplified from ear genomic DNA (first and second biopsy from 1 and 3 week old animals, respectively). Precise homologous recombination with the donor vector and therefore introduction of three stop codons as well as TfiI site insertion was detected by sequencing in this founder animal. Stop codon TGA underlined in red, TfiI restriction site underlined in green, endogenous start (ATG) and stop (TAG) codon underlined in black. **D.** Genotyping of *kcne5* mice by PCR. Exemplar agarose gel with *Kcne5*$^{+/+}$, *Kcne5*$^{+/-}$ and *Kcne5*$^{-/-}$ genotype.

**Supplementary Table S1:** Organ weights of C57BL/6 background *Kcne5*$^{+/+}$ and *Kcne5*$^{-/-}$ male mice (age of 4 month)

| | *Kcne5*$^{+/0}$(n=6) | *Kcne5*$^{-/0}$ (n=6) | p (Student`s T test) |
|---|---|---|---|
| HW (mg) | 158.0 ± 7.8 | 145.0 ± 6.4 | 0.233 |
| LW (mg) | 161.8 ± 4.7 | 149.8 ± 6.3 | 0.163 |
| HW/BW (mg/g) | 4.9 ± 0.1 | 4.7 ± 0.1 | 0.240 |
| LW/BW (mg/g) | 5.0 ± 0.1 | 4.9 ± 0.1 | 0.849 |
| BW (g) | 32.2 ± 1.1 | 30.6 ± 1.4 | 0.383 |

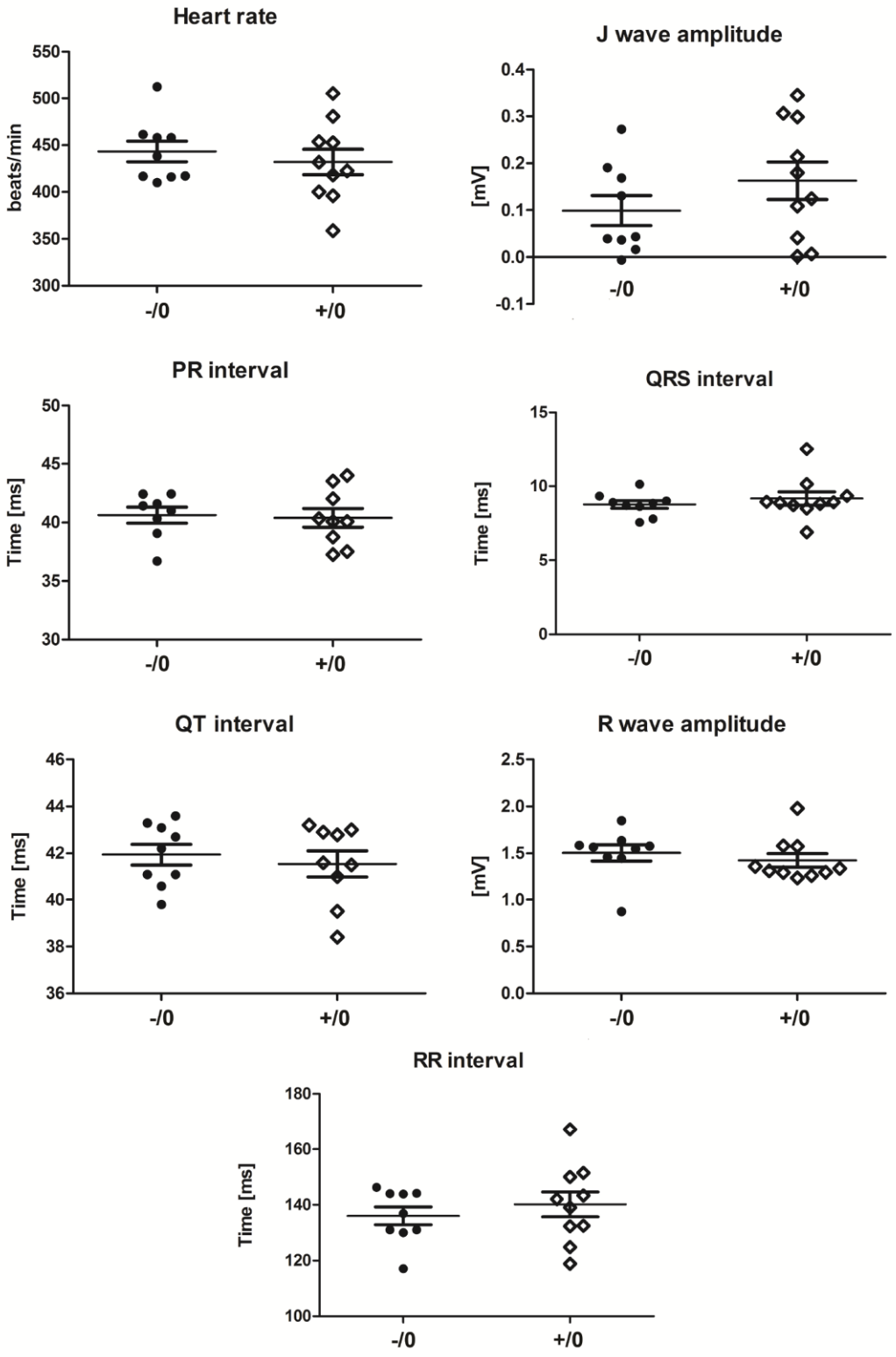
HW: heart weight, LW: Lung weight, BW: body weight; Values represent means ± SEM.

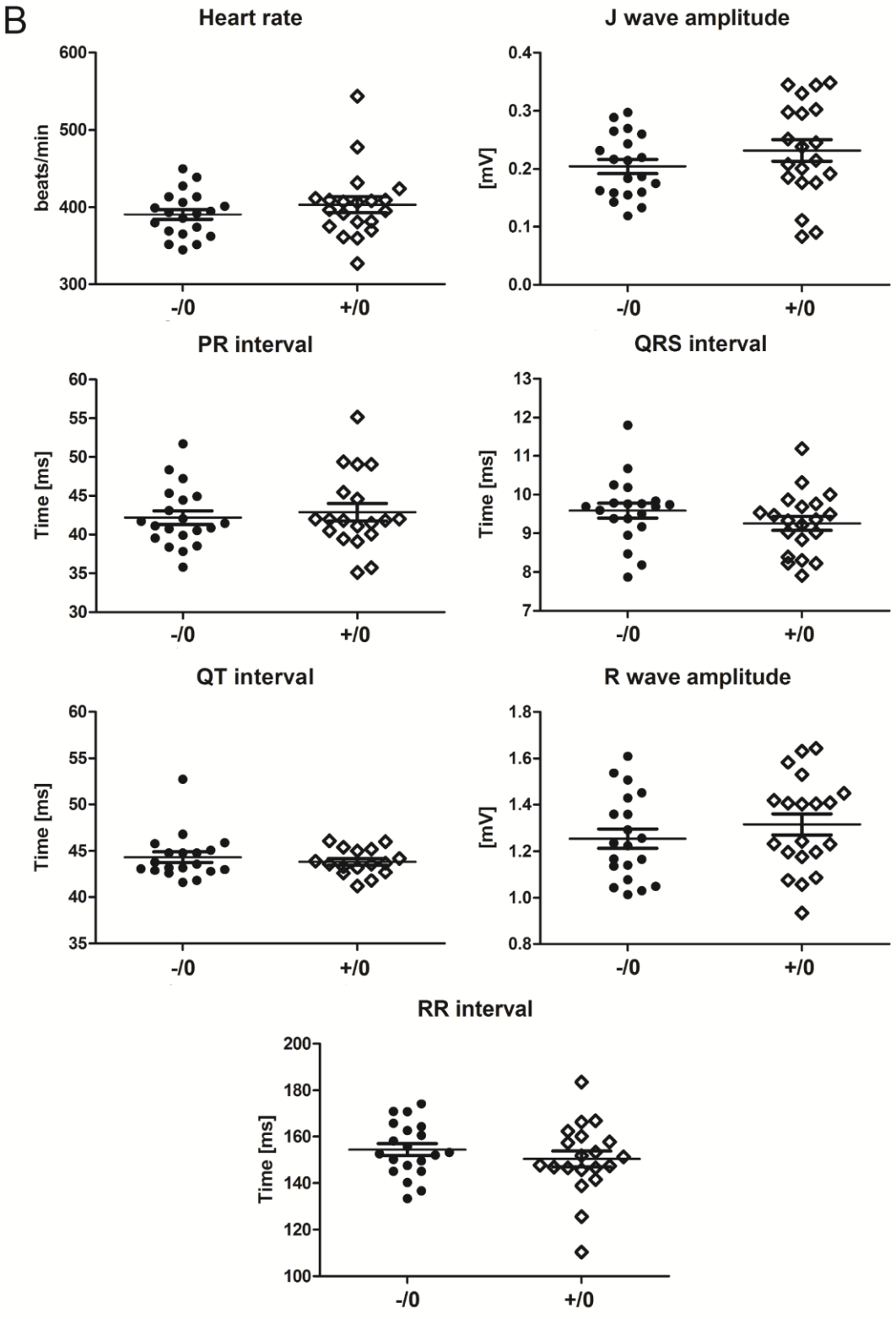
**Supplementary Table S2: Echocardiography** parameter of *Kcne5*$^{+/0}$ and *Kcne5*$^{-/0}$ male mice (ages 3-4 months)

| | *Kcne5*$^{+/+}$(*n*=7) | *Kcne5*$^{-/-}$ (*n*=7) | p (Student`s T test) |
|---|---|---|---|
| Heart rate (beats/min) | 440.43 ± 15.56 | 439.43 ± 18.00 | 0.821 |
| $IVS_d$ (mm) | 0.76 ± 0.04 | 0.69 ± 0.05 | 0.713 |
| $LVPW_d$ (mm) | 0.76 ± 0.05 | 0.71 ± 0.06 | 0.829 |
| $IVS_s$ (mm) | 1.14 ± 0.07 | 1.01 ± 0.07 | 0.616 |
| $LVPW_s$ (mm) | 1.07 ± 0.07 | 0.91 ± 0.08 | 0.526 |
| $LV_d$ (mm) | 4.67 ± 0.10 | 4.73 ± 0.17 | 0.469 |
| $LV_s$ (mm) | 3.50 ± 0.12 | 3.75 ± 0.22 | 0.365 |
| FS (%) | 25.10 ± 1.07 | 21.19 ± 1.87 | 0.307 |
| EF (%) | 52.50 ± 1.77 | 44.70 ± 3.63 | 0.280 |
| Stroke volume (µl) | 36.33 ± 3.46 | 31.67 ± 2.65 | 0.821 |
| Cardiac output (µl/min) | 15.90 ± 1.38 | 14.03 ± 1.55 | 0.890 |
| LV mass (mg) | 142.45 ± 8.87 | 130.57 ± 9.20 | 0.953 |
| HW/BW(mg/g) | 4.61 ± 0.23 | 4.50 ± 0.16 | 0.984 |
| BW (g) | 30.87 ± 0.95 | 28.90 ± 1.28 | 0.956 |

$IVS_d$ and $IVS_s$: intraventricular septum in diastole and systole, $LVPW_d$ and $LVPW_s$: left ventricular posterior wall in diastole and systole, $LV_d$ and $LV_s$: diameter of left ventricle in diastole and systole, FS: fractional shortening, EF: ejection fraction, HW: heart weight, BW: body weight; Values represent means ± SEM.

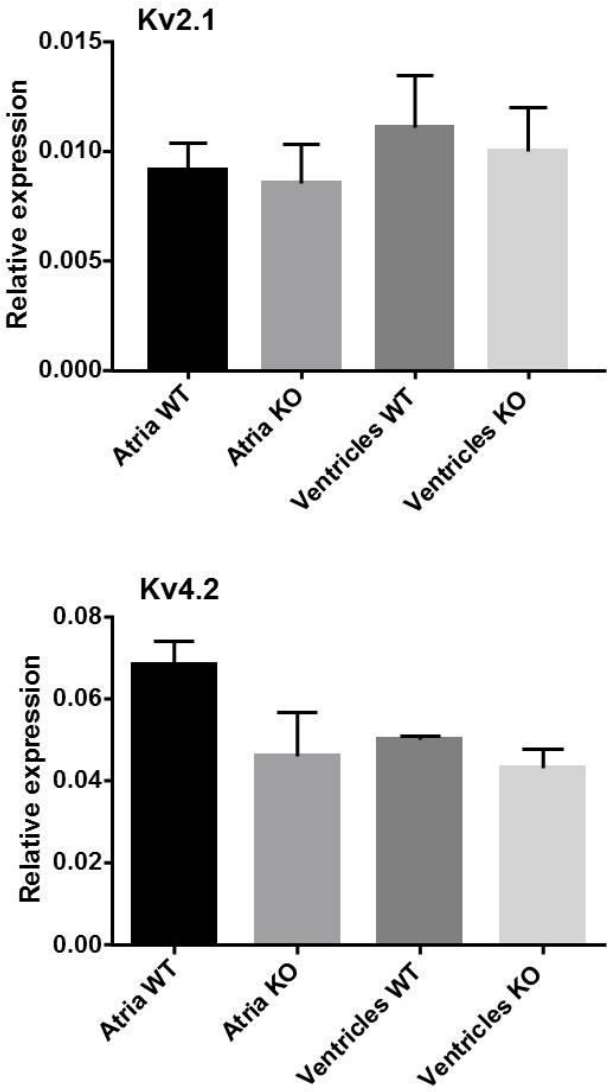
A


Heart rate
beats/min
550
500
450
400
350
300
-/0
+/0
J wave amplitude
[mV]
0.4
0.3
0.2
0.1
0.0
-0.1
-/0
+/0
PR interval
Time [ms]
50
45
40
35
30
-/0
+/0
QRS interval
Time [ms]
15
10
5
0
-/0
+/0
QT interval
Time [ms]
46
44
42
40
38
36
-/0
+/0
R wave amplitude
[mV]
2.5
2.0
1.5
1.0
0.5
0.0
-/0
+/0
RR interval
Time [ms]
180
160
140
120
100
-/0
+/0



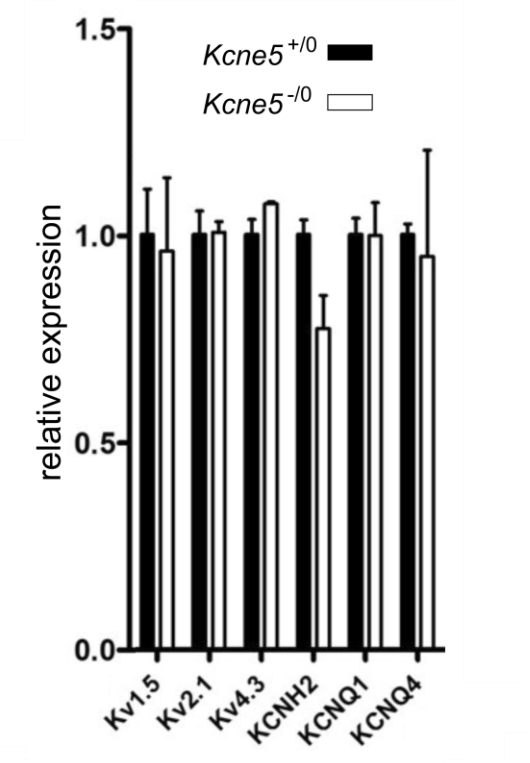

**Supplementary Figure S2: non-invasive body surface electrocardiography did not reveal altered parameters arising from *Kcne5* deletion.** Body surface ECG recordings were performed on mice anesthetized with isoflurane and an average ECG signal was generated (for more details see material and methods).

Parameters heart rate, RR interval, PR-interval, QRS interval, R wave amplitude, QT interval, and J wave amplitude are reported as mean±SEM. **A.** ECG analysis of *Kcne5*$^{-/0}$ male mice at the age ~5 months compared to age matched male control mice. Heart rate [bpm]: 443.2±11.1 (n=9) vs. 432.0±13.6 (n=10), RR interval [ms]: 136.0±3.2 (n=9) vs. 140.2±4.5 (n=10), PR interval [ms]: 40.6±0.7 (n=8) vs. 40.4±0.8 (n=9), QRS interval [ms]: 8.8±0.3 (n=9) vs. 9.2±0.5 (n=10), R wave amplitude [mV]: 1.51±0.09 (n=9) vs. 1.42±0.07 (n=10), QT interval [ms]: 41.9±0.4 (n=9) vs. 41.5±0.6 (n=9) and J wave amplitude [mV]: 0.10±0.03 (n=9) vs. 0.16±0.04 (n=10). Data were analyzed by unpaired Student´s t-test with Welch's correction, no statistically significant changes were observed. **B.** Comparing the ECG analysis between ~8 months old *Kcne5*$^{-/0}$ male mice and age matched male control mice. Heart rate [bpm]: 390.6±6.5 (n=20) vs. 403.3±10.1 (n=20), RR interval [ms]: 154.4±2.6 (n=20) vs. 150.4±3.4 (n=20), PR interval [ms]: 42.2±0.9 (n=20) vs. 42.9±1.1 (n=19), QRS interval [ms]: 9.6±0.2 (n=20) vs. 9.3±0.2 (n=20), R wave amplitude [mV]: 1.25±0.04 (n=20) vs. (1.31±0.05 n=20), QT interval [ms]: 44.3±0.6 (n=19) vs. 43.8±0.4 (n=16) and J wave amplitude [mV]: 0.20±0.01 (n=20) vs. 0.23±0.02 (n=20). No statistically significant differences were observed when analyzed with an unpaired Student´s t-test with Welch's correction.



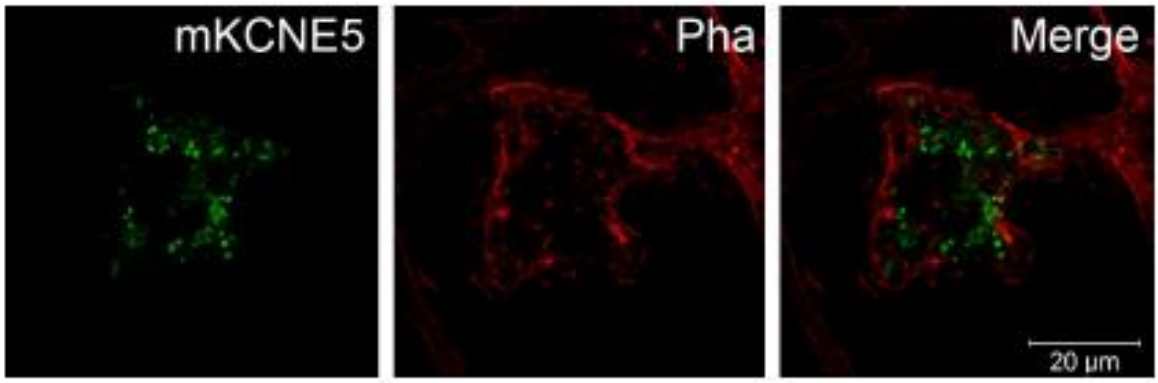

**Supplementary Figure S3: *Kcne5* deletion does not alter $K_V$ α subunit transcript expression in mouse hearts.**

**A.** Quantification of $K_V$2.1 (upper panel) or $K_V$4.2 (lower panel) transcript expression in atria and ventricles of male 129/SvEv-C57BL/6 background mice ($n$ = 3) by real-time qPCR, normalized to reference genes ACTB and GAPDH. Data presented as mean ± SEM. No statistically significant differences were observed between genotypes.

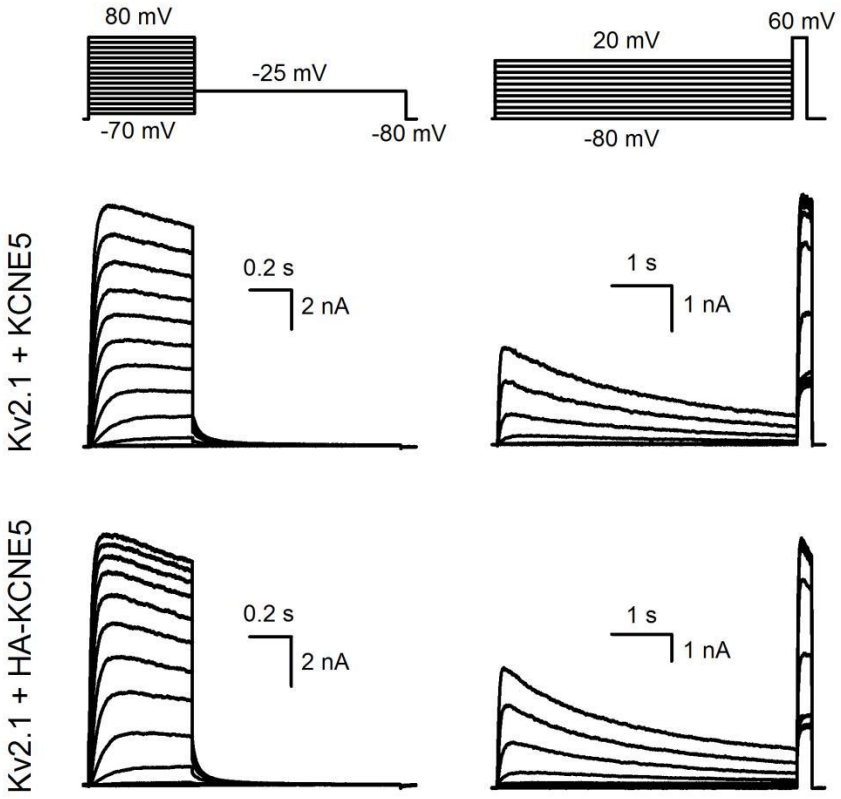



**Supplementary Figure S3: *Kcne5* deletion does not alter $K_V$ α subunit transcript expression in mouse hearts.**

**B.** Quantification of $K_V$ α subunit transcript expression in ventricles of male C57BL/6NCrl background mice (*n* = 3) by real-time qPCR, normalized to GAPDH. Data presented as mean ± SEM. No statistically significant differences were observed between genotypes.




**Supplementary Figure S4: Mouse KCNE5 subunits traffic similarly to human KCNE5 subunits.** The mouse clone of KCNE5 (mKCNE5) was found in similar dense clusters and vesicles as the human KCNE5 clone (see figure 6A) when singly expressed in HL-1 cells. Images were acquired using laser confocal microscopy. Merged image is found in the right column. Phalloidin (Pha) was used as a membrane maker.




**Supplementary Figure S5:** Biophysical properties of Kv2.1 co-expressed with wild type or HA-tagged KCNE5. Representative current recordings to determine the activation (left panel) and inactivation (right panel) properties of Kv2.1 + KCNE5 (middle row) and Kv2.1 + HA-KCNE5 (bottom row). The applied pulse protocols are given on top. No significant differences could be detected between wild type and HA-tagged KCNE5.