Data S1. Supplementary methods

Electrocardiography

Surface ECG was performed on WT- and *Kcnh2* heterozygous rats which were anaesthetized by isoflurane. Rats were placed in the anesthesia box, the oxygen flow rate was adjusted to 1 l/min, and isoflurane was adjusted to 3%. Rats were anaesthetized with a 1:1 mixture of isoflurane and oxygen. Once the rats lost the righting reflex, they were placed on a heated operation table with a supine position and the subcutaneous leads were placed in the conventional lead II position according to the instructions of the Power Lab Support apparatus (ECG Analysis Module; AD Instruments). Electrocardiograph signals were recorded for 30 min, and the value of electrocardiographic waves, the duration and intervals were analyzed using LabChart Pro software (AD Instruments). QTc was calculated using normalized Bazett's formula (1).

Echocardiography

Animal anesthesia was performed as *Electrocardiography*. When losing the righting reflex, the rats were laid supine on a heated platform, and a nosecone was connected to keep the rats anaesthetized under the condition of 1.5-2.0% isoflurane. An echocardiographic apparatus was used to record the heart beating from WT and *Kcnh2* heterozygous rats, respectively.

Whole-cell patch-clamp

The conventional whole-cell patch-clamp technique was used to record I_{Kr} and I_{Ks}. Procedures of the whole-cell patch-clamp method and Cs-carried I_{Kr} recording were performed as described previously (2). A regular pulse protocol was used to record Cs-I_{Kr} currents, which were elicited from a holding potential of -80 mV to various prepulse potentials of -70 to 70 mV for 1 s and repolarized to -70 mV to evoke outward Cs-I_{Kr} tail currents. The pipette solution contained (in mM) CsCl, 135; EGTA, 10; magnesium-ATP, 5 and HEPES, 10 (pH 7.2 with CsOH). The external solution contained (in mM) CsCl, 135; $MgCl_2$, 1; glucose, 10; and HEPES, 10, nimodipine 10 μM, (pH 7.4 with CsOH). All experiments were performed at room temperature (24-25˚C) using an Axopatch 700B amplifier (Molecular Devices, LLC.). The electrical signals were sampled at 2.5-10 kHz, filtered at 1 kHz using a low-pass filter, and digitized with an A/D converter (Digidata 1322; Axon Instruments). The pClamp software (version 10.2; Molecular Devices, LLC.) was used to generate voltage-pulse protocols and to acquire and analyze the data.

References

1. Kmecova J and Klimas J: Heart rate correction of the QT duration in rats. Eur J Pharmacol 641: 187-192, 2010.
2. Guo J, Massaeli H, Li W, Xu J, Luo T, Shaw J, Kirshenbaum LA and Zhang S: Identification of IKr and its trafficking disruption induced by probucol in cultured neonatal rat cardiomyocytes. J Pharmacol Exp Ther 321: 911-920, 2007.

Figure S1. A male chimaera was generated by injecting the rESCs into E4.5 blastocysts. The agouti coat color indicates that *Kcnh2* gene-targeted cells are present in the chimaera. rESCs, rat embryonic stem cells.

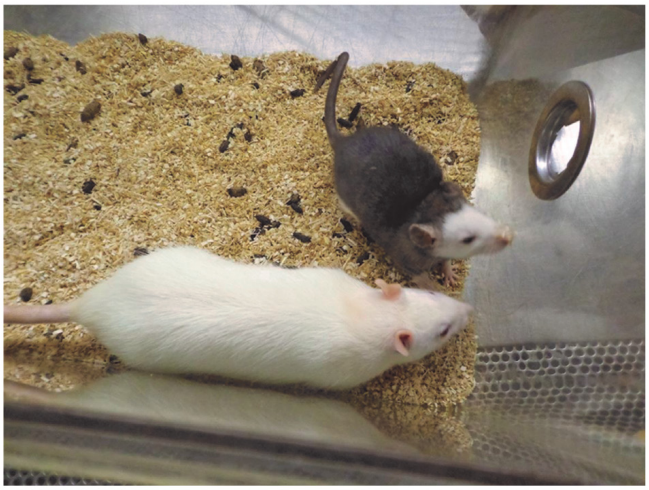

Figure S2. The morphology of *Kcnh2*$^{-/-}$ embryo. The *Kcnh2*$^{-/-}$ embryo is not visible at (A) E15 and (B) E18. Necrosis can be identified in the placenta of *Kcnh2*$^{-/-}$ embryo. WT, wild-type; H$^{+/-}$, *Kcnh2* heterozygote; H$^{-/-}$, *Kcnh2* homozygote.

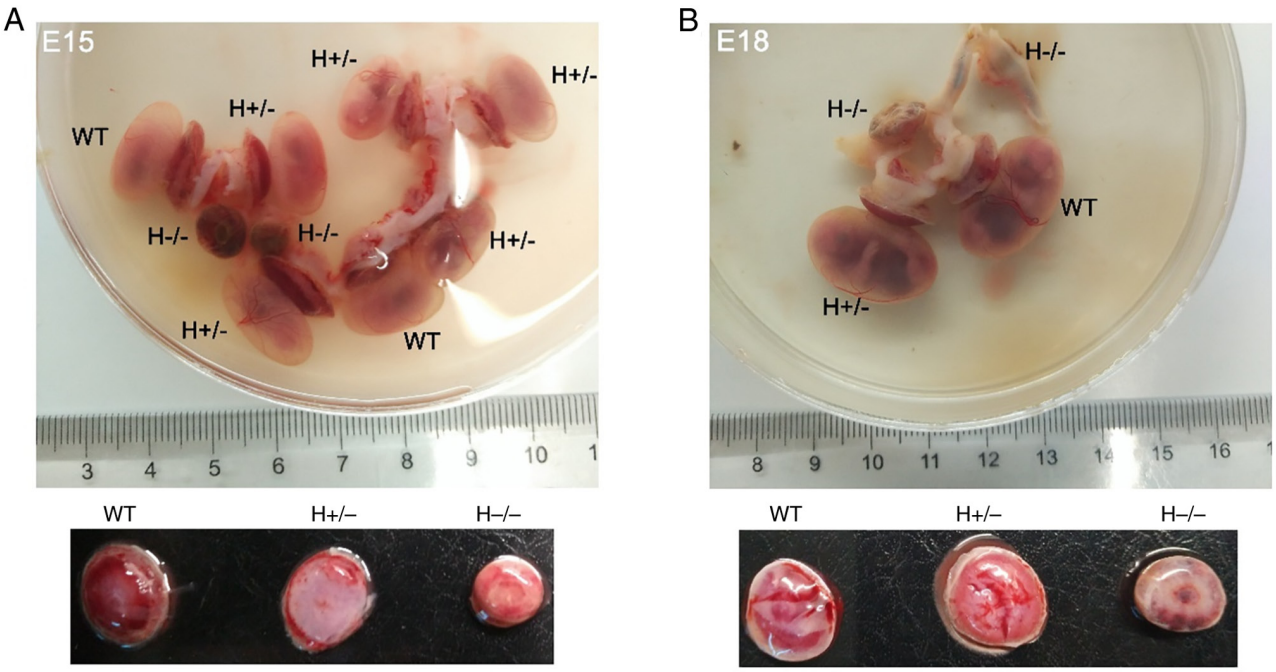

Figure S3. The original uncropped blots images of KCNH2. No variant blots were detected in the *Kcnh2*$^{-/-}$ rats. KCNH2, potassium voltage-gated channel subfamily H member 2.

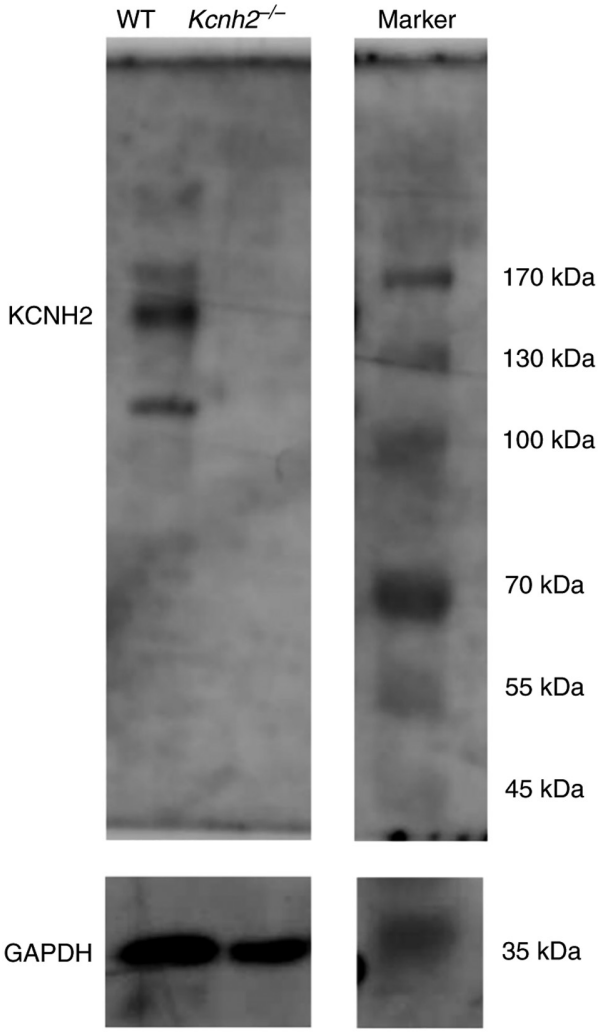

Figure S4. Immunofluorescence of ERG in the early vascular development in *Kcnh2*$^{-/-}$ rat embryos. No significant changes were seen in WT and *Kcnh2*$^{-/-}$ embryos. WT, wild-type; H$^{-/-}$, *Kcnh2* homozygote.

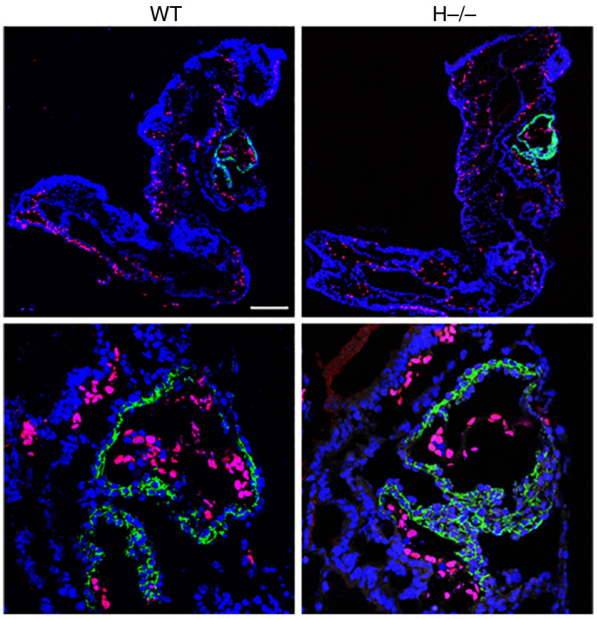

Figure S5. Expression status of cardiac markers in *Kcnh2* knockout E10.5 embryos. The reverse transcription-quantitative PCR analysis showed that the expression of most of the marker genes were significantly decreased. *P<0.05, **P<0.01, ***P<0.005. WT, wild-type; $H^{-/-}$, *Kcnh2* homozygote.

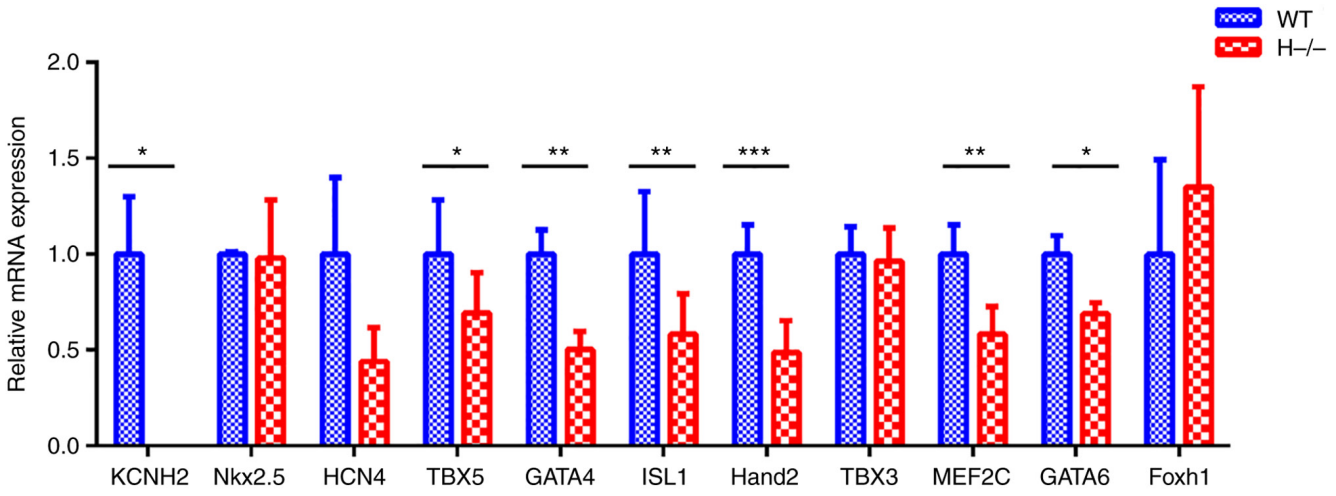

Figure S6. Effects of E4031 on embryo development. 8 mg/kg of E4031 was intraperitoneally injected into rat at i) E9.5 and embryos were harvested at E10.5, E11.5 or E12.5, respectively; ii) injected at E10.5 and embryos were harvested at E12.5(D); iii) injected at E11.5 and embryos were harvested at E13.5(E). With pharmacological inhibition of KCNH2 at E9.5, the (A) E10.5 embryos were normal and the (B and C) E11.5 and E12.5 embryos represented a similar body and heart size or even abnormally developed, which is similar with the KCNH2 knock out rat phenotype. Pharmacological inhibition of KCNH2 at (D and E) E10.5 or E11.5 cause embryonic lethality, which partly recapitulate the KCNH2 knock out phenotype. The data suggest an essential role of KCNH2 as a typical ion channel in early development of rat. Arrows indicate absorbed embryos shown on the right; scale bar, 500 μm. E4031, injection of E4031 on the indicated date; Exp, experiments for embryo dissection.

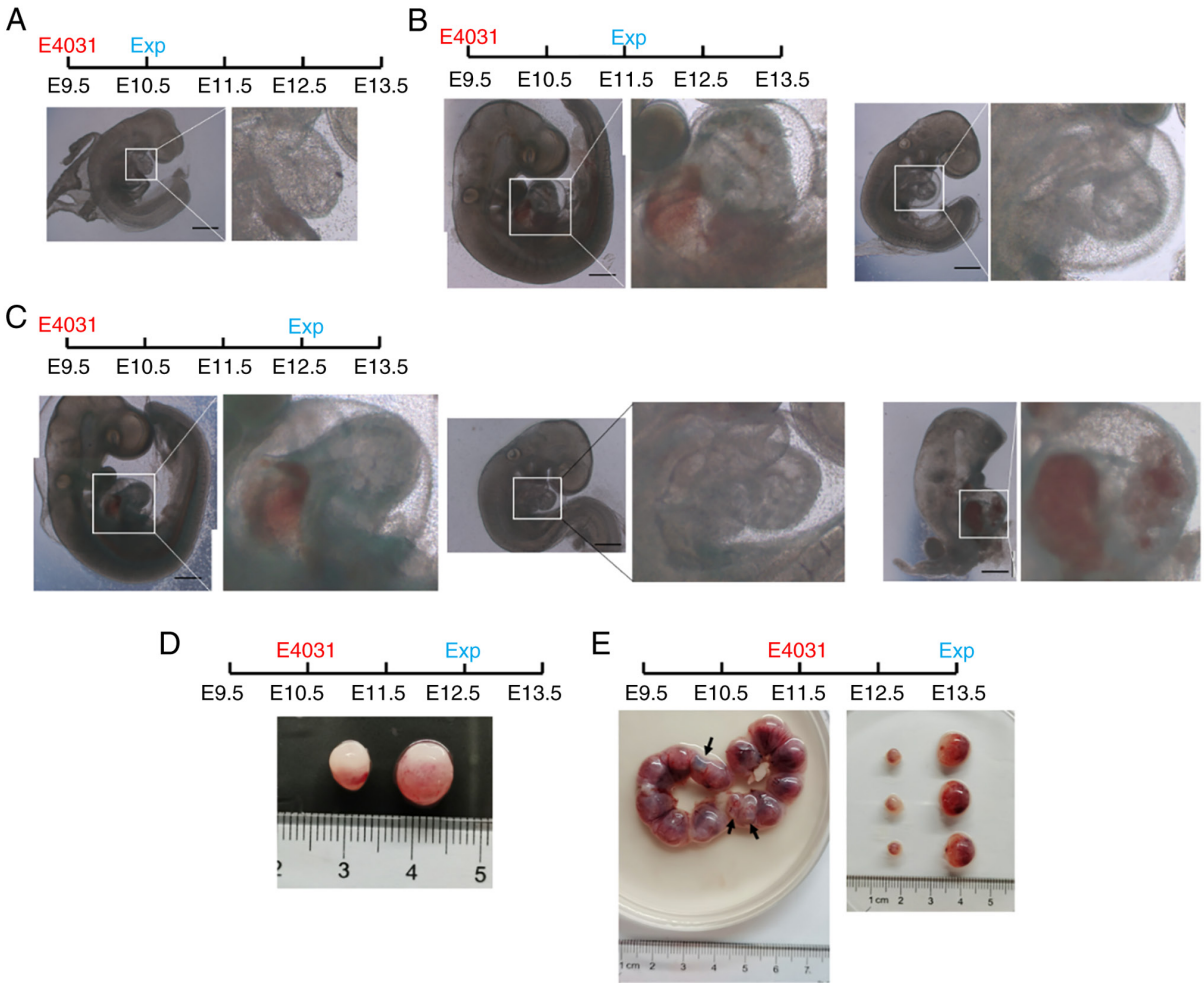

Figure S7. The expression status of KCNH2, CKMT2 and MYH7 in the single-cell dataset. (A) UMAP plots showing the expression of KCNH2 in different cardiomyocyte types. (B) The expression of CKMT2 along the trajectory. (C) The expression of MYH7 along the trajectory. KCNH2, potassium voltage-gated channel subfamily H member 2; UMAP, uniform manifold approximation and projection.

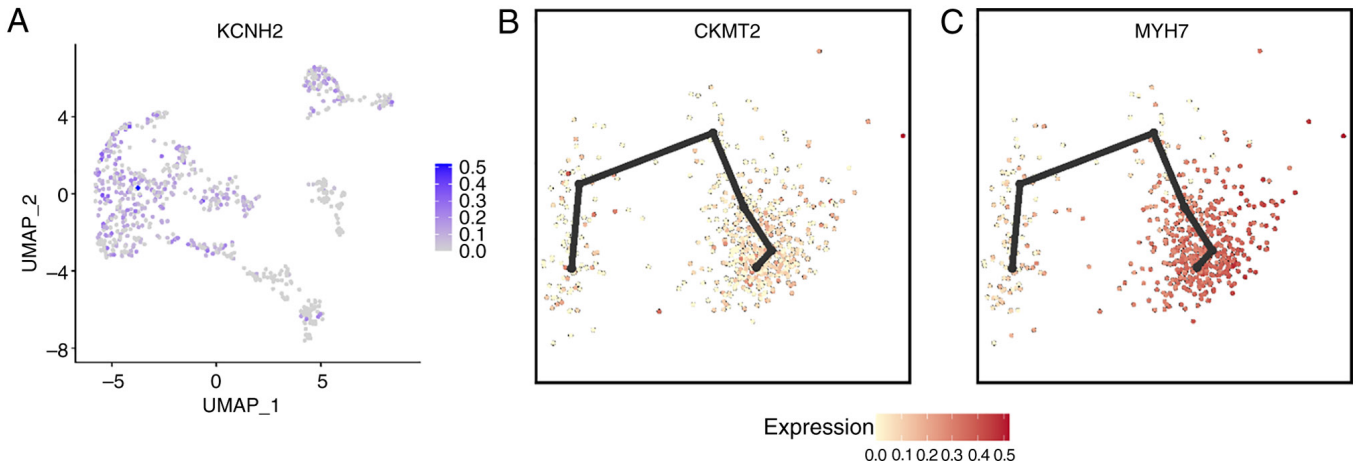

Figure S8. The expression of integrin β1 mRNA in *Kcnh2*$^{-/-}$ embryos. The expression of integrin β1 is not significantly changed in WT and *Kcnh2*$^{-/-}$ embryos. WT, wild-type; H$^{-/-}$, *Kcnh2* homozygote.

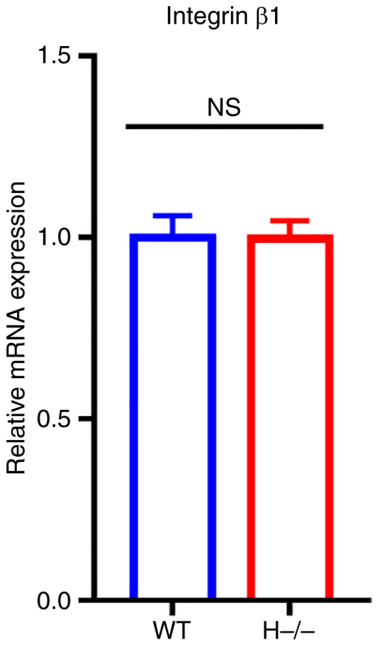

Figure S9. The expression of p-CaMKII and CaMKII in *Kcnh2*$^{-/-}$ in the E10.5 embryos. Upregulation of p-CaMKII and CaMKII was seen in WT and *Kcnh2*$^{-/-}$ embryos by (A and B) immunofluorescence and (C) western blotting. WT, wild-type; H$^{-/-}$, *Kcnh2* homozygote; p- phosphorylated.

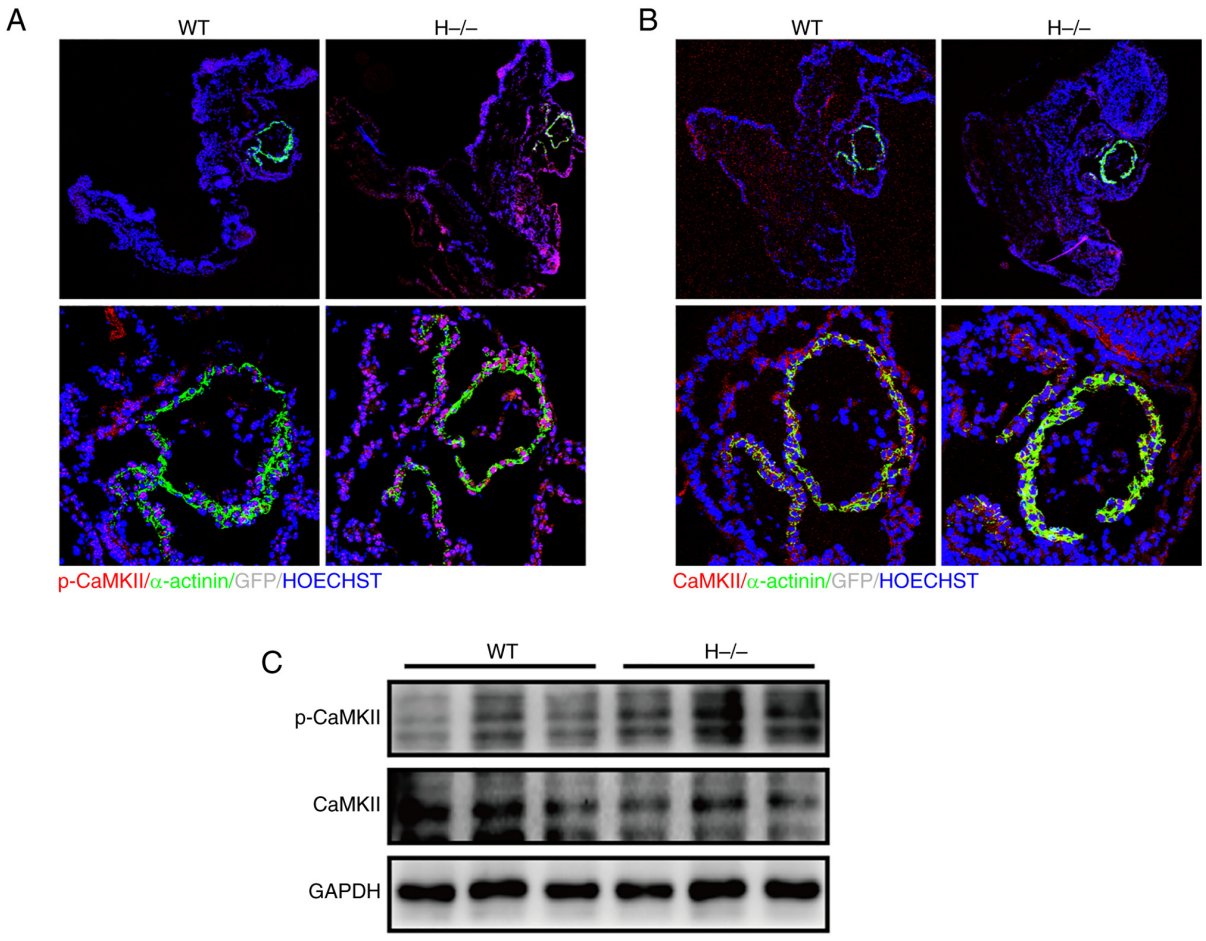

Figure S10. Electrophysiological differences in WT and *Kcnh2*$^{+/-}$ rat cardiomyocytes. Traces from the WT and *Kcnh2* heterozygote cardiomyocytes were shown on the left. Mean current-voltage relationships of the peak tail currents were shown on the right. No significant difference in the current between WT and *Kcnh2* heterozygote rats. WT, wild type; H$^{+/-}$, *Kcnh2* heterozygote.

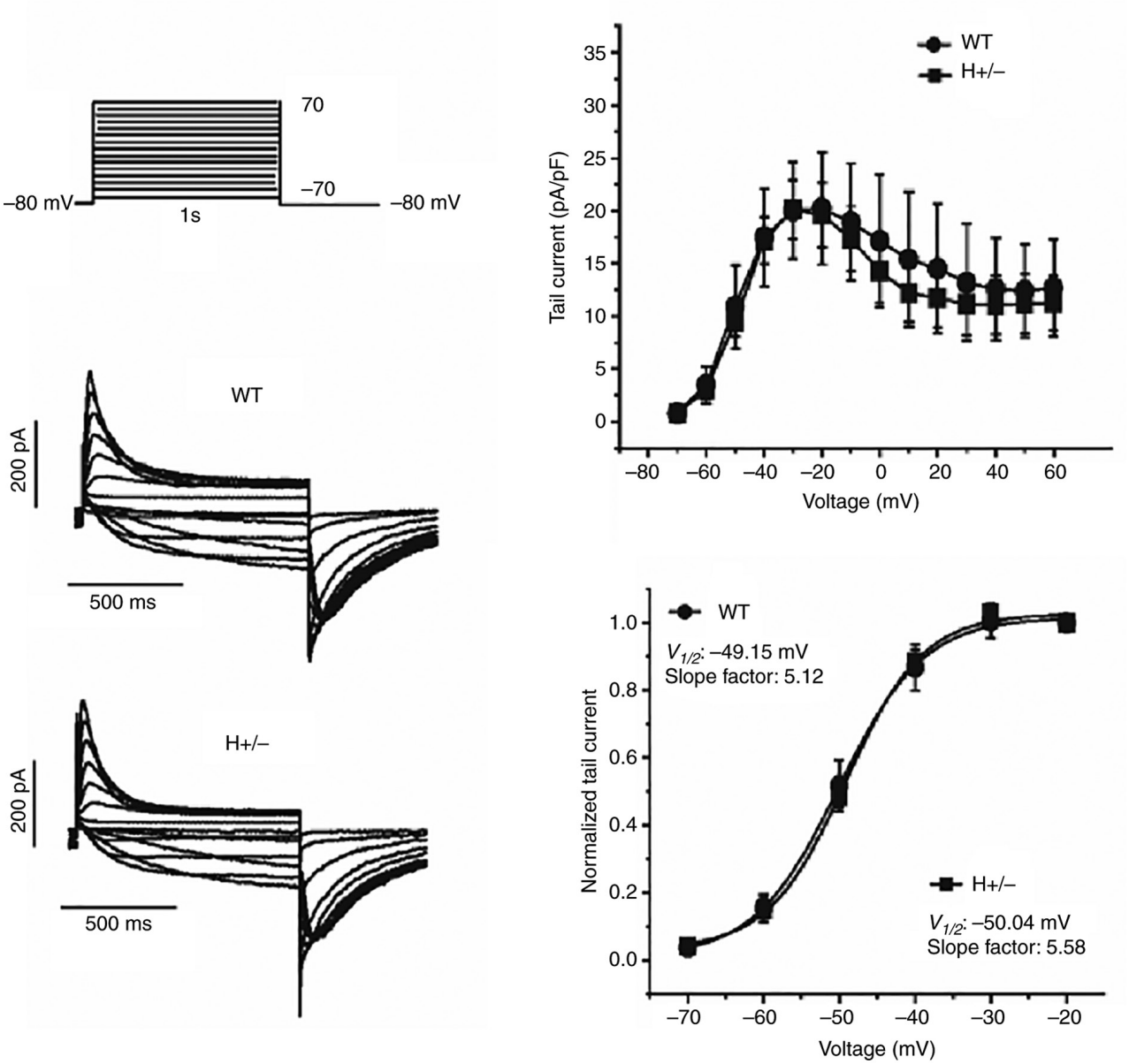

Figure S11. Heart function and structure of WT and *Kcnh2*$^{+/-}$ rats. (A and B) Echocardiography analysis for WT and *Kcnh2* heterozygous rat at ages of 1, 2 and 4 months. (C) Size change of the heart for WT and *Kcnh2*$^{+/-}$ rat at different stages. (D) The heart section of WT and *Kcnh2*$^{+/-}$ rat at different stages. No significant change was identified in WT and *Kcnh2*$^{+/-}$ embryos (scale bar, 2,000 μm). Data are represented as mean ± SD (n=3-5). EF, ejection fraction; FS, fractional shortening; IVS, interventricular septal thickness; LV Vol, left ventricular volume; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; WT, wild type; H$^{+/-}$, *Kcnh2* heterozygote; HW, heart weight; BW, body weight.

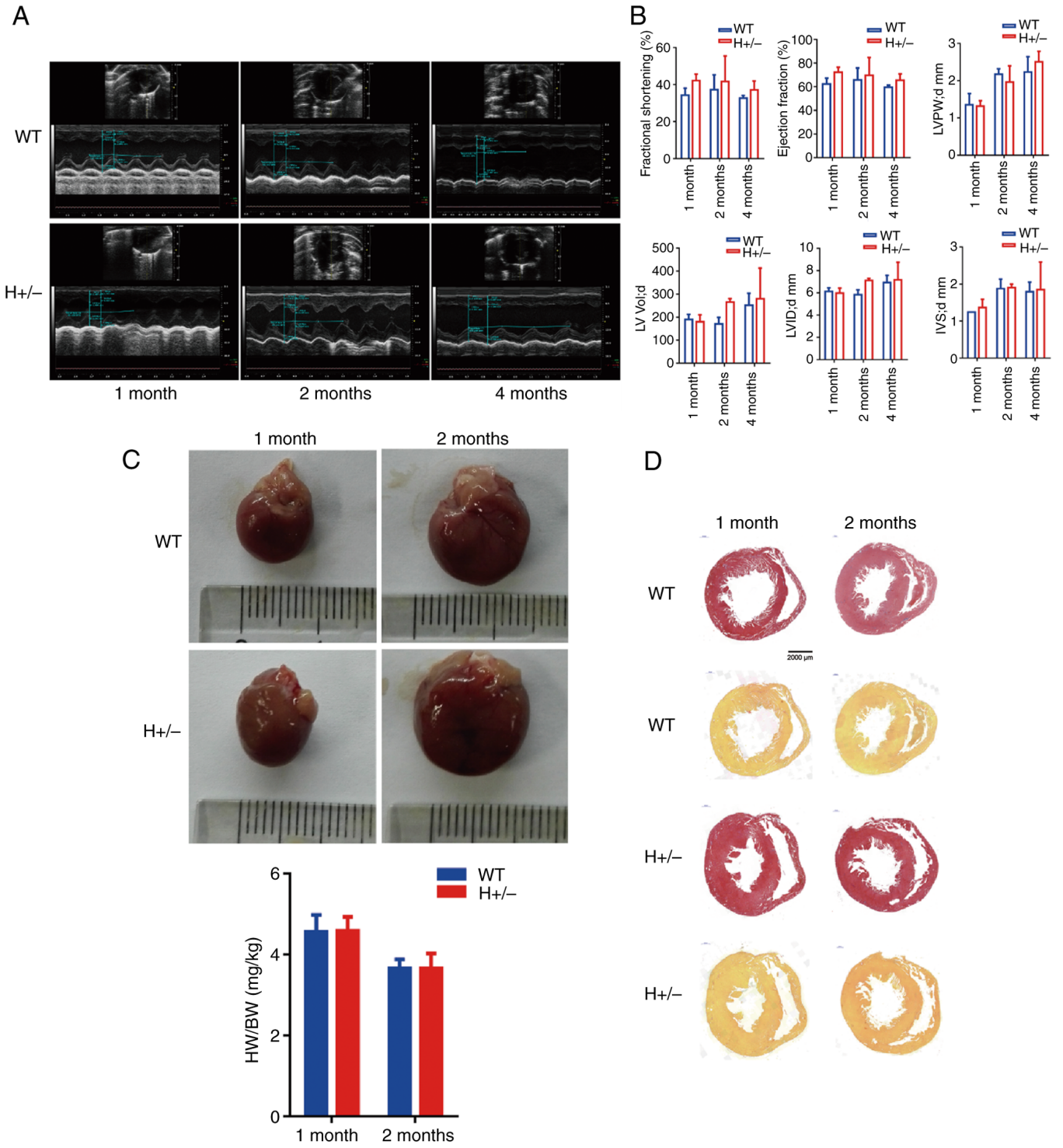

Figure S12. Electrophysiological change of WT and *Kcnh2*$^{+/-}$rats. (A) The change of quantification of T-wave interval for 1, 2- and 4-months old. (B) Restoration of ECG parameters in *Kcnh2* heterozygote rat treated with propranolol. Quantitative analysis showed the QTc interval, HR and T-Wave interval of *Kcnh2*$^{+/-}$ rat were fully restored after treatment with propranolol. The data are represented as mean ± SD (n=3-5); **P<0.01, ***P<0.005 vs. WT. ns, not significant; WT, wild type; H$^{+/-}$, *Kcnh2* heterozygote; HR, heart rate; H$^{+/-}$ + Pro, *Kcnh2* heterozygote after injection with propranolol (10 mg/kg).