## Materials and Methods:

### Inducible Nkx2-5 knockout mice

Floxed-Nkx2-5 homozygous mice (Nkx2-5$^{flox/flox}$)[1] were bred with transgenic mice carrying the Cre-ER$^{TM}$ gene under CMV promoter, Tg(CMV-Cre-ER$^{TM}$).[2] Subsequent matings between offsprings generated Nkx2-5$^{flox/flox}$/Tg(CMV-Cre-ER$^{TM}$)(flox/flox/Cre), Nkx2-5$^{wild/wild}$/Tg(CMV-Cre-ER$^{TM}$) (wild/wild/Cre) and Nkx2-5$^{flox/flox}$ (flox/flox). For perinatal deletion of the floxed-Nkx2-5 genes, a single injection of tamoxifen (0.5-1 mg/g body weight, ip) was administered to pregnant mice on gestation day 19. Male Tg(CMV-Cre-ER$^{TM}$)[2] were also crossed to females that carried the Rosa reporter allele, R26R[3]. All animal care protocols fully conformed to the Association for the Assessment and Accreditation of Laboratory Animal Care, with approvals from the University of Florida Institutional Animal Care and Use Committees.

### Southern, Northern and Western blotting and immunostaining

Southern and Northern blot analyses were performed using the following probes: Nkx2-5 5' genomic probe, NotI-ClaI fragment of mouse Nkx2-5 genomic DNA; Nkx2-5 coding probe, PflMI-EcoRI fragment of mouse Nkx2-5 cDNA; Cre-recombinase probe, EcoRI-BamHI fragment of pCAGGS-nls-Cre plasmid (gift from Dr. H. Xin); RT-PCR products amplified by the following primer sets: KcneI/minK (476 bp, F', 5'- CTTGACGCCCAGGATGAGC -3'; R, 5'- GGTGCCCCTACAATAAAGACTATGG -3'); HOD/HOP (703 bp, F', 5'- AGCAGACAGGCACCAGCATC -3'; R'- AAGGGAGCACAGGGAAGTGAAC -3'). Western blot analyses and immunostaining using frozen sections were performed with the following primary antibodies: anti-Nkx2-5 pAb[4], sarcomeric actinin (A7811, SIGMA), Nav1.5(□) (ASC-005, alomone labs), GAPDH (RDI-TRK5G4-6C5, Research Diagnostic Inc), RyR2 (MA3-916,

Affinity BioReagents), SERCA2a (sc-8095, Santa Cruz), phospholamban (05-205, Upstate). For detection of RyR and pentamer of phospholamban, protein samples were heated at 37ºC for 15 min before loading on SDS-PAGE gels.[5] Fluorescent microscopic images were obtained using ZEISS Axiovert200M with or without Apotome.

**Surface ECG recording and ultrasound imaging**

Recordings of six limb leads surface ECG (ADInstruments, Milford, MA) were performed without anesthesia as described previously with some modifications.[6] ECG recordings were analyzed using PowerLab software (ADInstruments).[6] M-mode ultrasound imaging of the left ventricle was obtained at the level of the papillary muscle from a parasternal window using an ultrasound biomicroscope with a single transducer with a frequency 55 MHz (VisualSonics, Tronto, Canada).

**Histological analyses**

X-gal staining of whole hearts and frozen sections were performed according to standard protocol.[7] Tissues were fixed in 4% paraformaldehyde/PBS overnight, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin/eosin or Masson's trichrome. Acetylcholine esterase staining in frozen tissue sections was performed as described previously with some modifications.[8] Whole mount acetylcholine esterase staining was performed with the incisions on right ventricular and atrial free wall, with addition of 0.1% Triton™ X-100 in all the solutions except for $Cu^{2+}$-glycine solution. After fixation with paraformaldehyde, tissue images were captured by a stereomicroscope (Nikon SMZ800) attached to a CCD camera (QImaging MicroPublisher3.3, BC, Canada), with scale bars present. The same magnifications and zoom

were applied for the hearts dissected from flox/flox or flox/flox/Cre mice at the same ages. Digitalized AV nodal surface size was measured using NIH Image software.

**Gene expression profiling**

Total RNA pooled from 3-4 hearts of postnatal day 4 (PD4) flox/flox or flox/flox/Cre mice with tamoxifen administration was analyzed in the CodeLink Bioarrays followed by CodeLink and GeneSpring software (Genus Biosystems, Northbrook, IL). Four sets of relative expression values between flox/flox and flox/flox/Cre were averaged and are listed.

**Measurement of sodium current and simultaneous recording of cardiac contraction and $Ca^{2+}$**

After genotyping, neonatal ventricular cardiomyocytes were isolated from flox/flox or flox/flox/Cre mice side by side at PD3, cultured for 4-5 days before measuring sodium currents (equivalent to PD7-8). Sodium currents were ($I_{Na}$) obtained by voltage clamp recordings in the whole cell patch clamp configuration at room temperature (~22°C). Myocytes were bathed in low sodium buffer containing, in mmol/L: NaCl 10, CsCl 80, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, 4-aminopyridine 10, tetraethylammonium-Cl 30, dextrose 10, CdCl 0.1; and nifedipine 5 μM with an osmolarity of 290 mOsm and pH of 7.4 adjusted with CsOH. Recordings were obtained using Molecular Devices Axon 200A amplifier (Sunnyvale, CA) filtered at 2 kHz and digitized using Molecular Devices, Digidata 1322A, at 10 kHz. Data were acquired using Molecular Devices PClamp 9.0 software. Myocytes were impaled with Drummond Wiretrol II capillary pipettes (Drummond Scientific Company, Broomall, PA) pulled with Sutter instrument P-97 horizontal pipette puller and polished with Narishige MF-830 microforge with series resistance of 0.5 to 1.5

mΩ when filled with solution containing, in mmol/L: CsOH 105, glutamic acid 105, tetraethylammonium-Cl 20, HEPES 10, $Cs_2$-EGTA 5, MgATP 5, NaGTP 0.1 with osmolarity of 285 and pH adjusted to 7.2 with CsOH. A -9.7 mV correction for liquid junction potential, between the patch pipette and bath solution, was set upon access to the cell cytosol. Cell capacitance and input series resistance were electronically compensated to approximately 90 percent using online circuitry. Leak currents were subtracted online with P/4 protocol.

Data analysis was performed using Molecular devices Clampfit 9.0, Microsoft Excel, and GraphPad Prism 4.0 (San Diego, CA). $I_{Na}$ current density was derived by dividing current amplitude from each recording by their respective cell capacitance obtained from amplifier upon compensation. Experiments that showed inadequate voltage control, such as space clamp error or inappropriate steep increase in current amplitude in the negative slope of current voltage relation, were discarded. Steady state activation curves were generated from data used to obtain current voltage relation plots fitted to a Boltzman equation: $I/I_{Max} = 1/[1+\exp(V - V_{1/2} / K_V)]$ where $I/I_{Max}$ is the current expressed as fraction of maximal current; $V_{1/2}$ is the voltage to obtain half maximal current and $K_{mV}$ is the slope factor.

Cardiomyocytes were isolated from 3 weeks old flox/flox or flox/flox/Cre mice. Only rod-shaped cardiomyocytes with staircase ends, clear cross striations and surface membranes free from blebs were studied for simultaneous measurements of cell shortening and intracellular free calcium cardiomyocyte contraction and simultaneous $Ca^{2+}$ measurement as described previously.[9]

**Real-time reverse transcriptase (RT)-PCR**

Real-time RT-PCR was performed using the inventoried Taqman Gene Expression Assays (Applied Biosystems): Scn5a, Mm00451971_m1; RyR2, Mm00465877_m1; T-type Ca channel 1G, Mm00486549_m1; T-type Ca channel 1H, Mm00445369_m1; Scn1a, Mm00450580; Scn3a, Mm00658167; Scn4a, Mm00500103; Scn7a, Mm00801952; Scn8a, Mm00488110; Scn9a, Mm00450762; Scn1b, Mm0041210. Data were normalized with beta-actin expression (product No, 4352933E). Duplicate experiments were averaged.

**Reporter assays**

C57BL/6 genomic DNA or BAC clone (Invitrogen, RPC 123.C, Clone ID: 190M7) was subjected to PCR for amplification of Scn5a genomic DNA fragments using specific primers: (fragment -736 to +119, F, 5'-TGGCGGTGTGTTTGATTTCAG-3'; R, 5'-GGGCTCGGTTCGGCGTAG-3'), (fragment -2308 to +119, F, 5'-GGCTGGAAGTGGTGACATTAGAG-3'; R, 5'-TGGCGGTGTGTTTGATTTCAG-3'), (fragment -9282 to -8098, F, 5'- TGGCAACCGCAGAACGAC -3'; R, 5'-CCCTCCTCCCCGCAATCAC -3'), (fragment -10419 to -9917, F, 5'-TGTGTGGGTGTATGGGTGACC-3'; R, 5'-GGAGGTAGTGCGTCTGTTCCTATC-3'), (fragment -17405 to -16448, F, 5'-ATGTTCCTGGCATAACCCGAG-3'; R, 5'-AAACCCTTTCCTCCCCCG-3'), (fragment -27145 to -26589, F, 5'-CCAAGCCACACTCTCAGGTCAC-3'; R, 5'-GCAAGCACAGGGGGACTGG-3'). PCR fragments were cloned into pCR-Blunt II-TOPO or pCR2.1 vector (Invitrogen), sequenced, and inserted into pGL3 basic plasmid (Promega) using appropriate restriction enzyme sites. Nkx2-5 non-binding mutant of -736 to +119 bp fragments were generated by PCR using two primers; F,

5'-
TGTGGTTCTGGGTGTCCCGGTGTCAGTGTGTCAATGGATGTGTCTCTGGGTCACGTG
GTA-3'; R, 5'-
TACCACGTGACCCAGAGACACATCCATTGACACACTGACACCGGGACACCCAGAAC
CACA-3'.

10T1/2 fibroblast cells cultured in six-well plates were cotransfected with 3 μg of luciferease reporter constructs, 1 μg of pcDNA3 or pcDNA3-Nkx2-5 expression plasmid and 0.5 μg of Rous sarcoma virus β-galactosidase construct using the calcium phosphate method as described previously.[10]

**Statistical analyses**

Results among groups except for analyses of sodium currents (see above) were compared using ANOVA and Fisher's PLSD or Bonferroni's post-hoc test (StatView version 5.01).

**References:**

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**Table S1. The six most reduced ion channels in Nkx2-5 knockout hearts by gene expression profiling.**

**PD4**

| Protein | Gene | UniGene No. | fold difference KO vs. control (mean ± S.E., n=4) |
|---|---|---|---|
| calcium channel, voltage-dependent, T type, alpha 1H subunit | Cacna1h | Mm.268378 | 0.321 ± 0.012 |
| potassium voltage-gated channel, Isk-related subfamily, member 1 | Kcne1 | Mm.299425 | 0.367 ± 0.002 |
| calcium channel, voltage-dependent, T type, alpha 1G subunit | Cacna1g | Mm.29585 | 0.518 ± 0.018 |
| sodium channel, voltage-gated, type V, alpha polypeptide | Scn5a | Mm.103584 | 0.745 ± 0.009 |
| ryanodine receptor 2, cardiac | Ryr2 | Mm.239871 | 0.766 ± 0.057 |
| gap junction membrane channel protein alpha 5 (connexin 40) | Gja5 | Mm.281816 | 0.767 ± 0.029 |

## Table S2. Expression of ion channel-related genes in Nkx2-5 knockout heart.

| Protein | Gene | UniGene No. | fold difference KO vs. control (mean ± S.E., n=4) |
|---|---|---|---|
| calcium channel, voltage-dependent, T type, alpha 1H subunit | Cacna1h | Mm.268378 | 0.321 ± 0.012 |
| potassium voltage-gated channel, Isk-related subfamily, member 1 | Kcne1 | Mm.299425 | 0.367 ± 0.002 |
| calcium channel, voltage-dependent, T type, alpha 1G subunit | Cacna1g | Mm.29585 | 0.518 ± 0.018 |
| sodium channel, voltage-gated, type V, alpha polypeptide | Scn5a | Mm.103584 | 0.745 ± 0.009 |
| ryanodine receptor 2, cardiac | Ryr2 | Mm.239871 | 0.766 ± 0.057 |
| gap junction membrane channel protein alpha 5 | Gja5 | Mm.281816 | 0.767 ± 0.029 |
| calcium channel, voltage-dependent, L type, alpha 1D subunit | Cacna1d | Mm.9772 | 0.780 ± 0.036 |
| Discs, large homolog 2 (Drosophila) | Dlg2 | Mm.257035 | 0.783 ± 0.043 |
| potassium inwardly rectifying channel, subfamily J, member 11 | Kcnj11 | Mm.333863 | 0.805 ± 0.025 |
| potassium intermediate/small conductance calcium-activated channel, subfamily N, member 1 | Kcnn1 | Mm.32074 | 0.812 ± 0.004 |
| chloride channel 2 | Clcn2 | Mm.177761 | 0.819 ± 0.140 |
| purinergic receptor P2X, ligand-gated ion channel, 7 | P2rx7 | Mm.42026 | 0.824 ± 0.019 |
| chloride intracellular channel 3 | Clic3 | Mm.44194 | 0.826 ± 0.035 |
| tenascin XB | Tnxb | Mm.290527 | 0.833 ± 0.021 |
| aquaporin 4 | Aqp4 | Mm.250786 | 0.839 ± 0.006 |
| transient receptor potential cation channel, subfamily V, member 3 | Trpv3 | Mm.347652 | 0.840 ± 0.060 |
| solute carrier family 8 (sodium/calcium exchanger), member 1 | Slc8a1 | Mm.265834 | 0.844 ± 0.075 |
| potassium voltage-gated channel, shaker-related subfamily, member 5 | Kcna5 | Mm.222831 | 0.849 ± 0.065 |
| potassium channel, subfamily T, member 2 | Kcnt2 | Mm.329016 | 0.850 ± 0.068 |
| potassium channel tetramerisation domain containing 5 | Kctd5 | Mm.28171 | 0.858 ± 0.026 |
| potassium channel, subfamily K, member 3 | Kcnk3 | Mm.332293 | 0.865 ± 0.028 |
| chloride intracellular channel 5 | Clic5 | Mm.37666 | 0.869 ± 0.058 |
| solute carrier family 8 (sodium/calcium exchanger), member 2 | Slc8a2 | Mm.241147 | 0.872 ± 0.013 |
| gap junction membrane channel protein alpha 3 | Gja3 | Mm.57207, Mm.393732 | 0.877 ± 0.059 |
| potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2 | Kcnn2 | Mm.411614 | 0.885 ± 0.089 |
| potassium voltage-gated channel, Isk-related subfamily, gene 4 | Kcne4 | Mm.24386 | 0.889 ± 0.046 |
| potassium channel tetramerisation domain containing 15 | Kctd15 | Mm.214380, Mm.407585 | 0.892 ± 0.024 |
| Potassium voltage gated channel, Shaw-related subfamily, member 2 | Kcnc2 | Mm.336242 | 0.897 ± 0.065 |
| channel-interacting PDZ domain protein, transcript variant 3 | Cipp | Mm.90218 | 0.903 ± 0.021 |
| Ryanodine receptor 1, skeletal muscle | Ryr1 | Mm.226037 | 0.910 ± 0.106 |
| chloride channel 5 | Clcn5 | Mm.254370 | 0.910 ± 0.121 |
| Kv channel-interacting protein 2 | Kcnip2 | Mm.213204 | 0.911 ± 0.013 |
| gap junction membrane channel protein beta 4 | Gjb4 | Mm.56906 | 0.914 ± 0.066 |
| potassium voltage-gated channel, shaker-related subfamily, member 4 | Kcna4 | Mm.142718 | 0.920 ± 0.068 |
| purinergic receptor P2X, ligand-gated ion channel 4 | P2rx4 | Mm.290884 | 0.921 ± 0.034 |
| potassium voltage-gated channel, subfamily Q, member 4 | Kcnq4 | Mm.249977 | 0.921 ± 0.029 |
| calcium channel, voltage-dependent, alpha 2/delta subunit 2 | Cacna2d2 | Mm.273084 | 0.921 ± 0.030 |
| purinergic receptor P2X, ligand-gated ion channel, 1 | P2rx1 | Mm.25722 | 0.924 ± 0.019 |
| glutamate receptor, ionotropic, kainate 5 (gamma 2) | Grik5 | Mm.2879 | 0.925 ± 0.040 |
| potassium channel, subfamily K, member 13 | Kcnk13 | Mm.312335 | 0.927 ± 0.047 |
| transient receptor potential cation channel, subfamily V, member 4 | Trpv4 | Mm.266450 | 0.929 ± 0.013 |
| transmembrane channel-like gene family 5 | Tmc5 | Mm.11068 | 0.929 ± 0.012 |
| potassium channel, subfamily K, member 16 | Kcnk16 | Mm.105571 | 0.932 ± 0.020 |

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|---|---|---|---|
| transient receptor potential cation channel, subfamily M, member 7 | Trpm7 | Mm.244705 | 0.933 ± 0.037 |
| calcium channel, voltage-dependent, beta 2 subunit | Cacnb2 | Mm.313930 | 0.933 ± 0.032 |
| sodium channel, voltage-gated, type IV, alpha polypeptide | Scn4a | Mm.432528 | 0.934 ± 0.038 |
| glutamate receptor, ionotropic, kainate 2 (beta 2) | Grik2 | Mm.332838 | 0.937 ± 0.052 |
| chloride channel calcium activated 1 | Clca1 | Mm.20897 | 0.937 ± 0.034 |
| calsenilin, presenilin binding protein, EF hand transcription factor | Csen | Mm.315292 | 0.945 ± 0.070 |
| gap junction membrane channel protein beta 6 | Gjb6 | Mm.25652 | 0.949 ± 0.022 |
| glutamate receptor, ionotropic, delta 2 | Grid2 | Mm.425327 | 0.950 ± 0.055 |
| potassium voltage-gated channel, subfamily H (eag-related), member 2 | Kcnh2 | Mm.6539 | 0.950 ± 0.037 |
| potassium voltage-gated channel, subfamily G, member 4 | Kcng4 | Mm.358699 | 0.951 ± 0.050 |
| potassium voltage-gated channel, subfamily Q, member 1 | Kcnq1 | Mm.356096 | 0.952 ± 0.020 |
| chloride channel 7 | Clcn7 | Mm.270587 | 0.954 ± 0.053 |
| Yip1 domain family, member 2 | Yipf2 | Mm.178115 | 0.954 ± 0.025 |
| sodium channel, voltage-gated, type III, alpha | Scn3a | Mm.330256 | 0.954 ± 0.053 |
| hydrogen voltage-gated channel | Hvcn1 | Mm.28804 | 0.955 ± 0.042 |
| potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 | Kcnn4 | Mm.9911 | 0.957 ± 0.108 |
| sodium channel, voltage-gated, type XI, alpha polypeptide | Scn11a | Mm.89981 | 0.957 ± 0.018 |
| Shugoshin-like 2 (S. pombe) | Sgol2 | Mm.339711 | 0.959 ± 0.036 |
| transient receptor potential cation channel, subfamily C, member 2 | Trpc2 | Mm.292904 | 0.967 ± 0.010 |
| amiloride-sensitive cation channel 4, pituitary | Accn4 | Mm.110790 | 0.967 ± 0.051 |
| chloride intracellular channel 6 | Clic6 | Mm.44747 | 0.968 ± 0.036 |
| transient receptor potential cation channel, subfamily C, member 5 | Trpc5 | Mm.328378, Mm.434419 | 0.968 ± 0.061 |
| gap junction membrane channel protein alpha 6 | Gja6 | Mm.123113 | 0.970 ± 0.022 |
| calcium channel, voltage-dependent, gamma subunit 7 | Cacng7 | Mm.214994 | 0.970 ± 0.138 |
| gap junction membrane channel protein alpha 12 | Gja12 | Mm.40016 | 0.971 ± 0.040 |
| hyperpolarization-activated, cyclic nucleotide-gated K+ 3 | Hcn3 | Mm.389461 | 0.972 ± 0.041 |
| two pore segment channel 2 | Tpcn2 | Mm.102235 | 0.974 ± 0.025 |
| gap junction membrane channel protein beta 2 | Gjb2 | Mm.390683 | 0.975 ± 0.045 |
| transmembrane channel-like gene family 6 | Tmc6 | Mm.286963 | 0.975 ± 0.063 |
| calcium channel, voltage-dependent, L type, alpha 1C subunit | Cacna1c | Mm.436656 | 0.976 ± 0.028 |
| ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 | Atp2a2 | Mm.227583 | 0.977 ± 0.031 |
| glutamate receptor, ionotropic, delta 1 | Grid1 | Mm.390745 | 0.979 ± 0.023 |
| potassium channel, subfamily K, member 4 | Kcnk4 | Mm.12894 | 0.981 ± 0.132 |
| olfactomedin 1 | Olfm1 | Mm.43278 | 0.981 ± 0.059 |
| chloride channel calcium activated 4 | Clca4 | Mm.290600 | 0.982 ± 0.024 |
| phospholamban | Pln | Mm.34145 | 0.984 ± 0.041 |
| trinucleotide repeat containing 6b | Tnrc6b | Mm.131328 | 0.986 ± 0.053 |
| chloride channel Kb | Clcnkb | Mm.24882 | 0.989 ± 0.068 |
| bone morphogenetic protein 10 | Bmp10 | Mm.57171 | 0.990 ± 0.093 |
| sodium channel, voltage-gated, type VI, alpha polypeptide | Scn7a | Mm.38127, Mm.389273 | 0.990 ± 0.050 |
| Kv channel-interacting protein 1 | Kcnip1 | Mm.252514 | 0.990 ± 0.013 |
| potassium inwardly-rectifying channel, subfamily J, member 14 | Kcnj14 | Mm.68170 | 0.994 ± 0.023 |
| K+ voltage-gated channel, subfamily S, 1 | Kcns1 | Mm.6217, Mm.439082 | 0.994 ± 0.063 |
| potassium voltage-gated channel, subfamily G, member 3 | Kcng3 | Mm.223506 | 0.994 ± 0.015 |
| transient receptor potential cation channel, subfamily C, member 4 associated protein | Trpc4ap | Mm.268304, Mm.375116 | 0.997 ± 0.032 |
| structure specific recognition protein 1 | Ssrp1 | Mm.219793 | 0.997 ± 0.054 |
| potassium voltage-gated channel, shaker-related subfamily, member 7 | Kcna7 | Mm.12955 | 0.999 ± 0.027 |

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|---|---|---|---|
| chloride channel 3 | Clcn3 | Mm.259751, Mm.25263 | 0.999 ± 0.021 |
| potassium inwardly-rectifying channel, subfamily J, member 6 | Kcnj6 | Mm.328720 | 0.999 ± 0.043 |
| potassium voltage-gated channel, shaker-related subfamily, member 2 | Kcna2 | Mm.56930 | 1.000 ± 0.020 |
| transient receptor potential cation channel, subfamily V, member 3 | Trpv3 | Mm.75196 | 1.000 ± 0.055 |
| potassium voltage-gated channel, shaker-related subfamily, beta member 3 | Kcnab3 | Mm.232472 | 1.002 ± 0.008 |
| potassium voltage gated channel, Shab-related subfamily, member 1 | Kcnb1 | Mm.387390, Mm.395669 | 1.006 ± 0.031 |
| potassium channel, subfamily K, member 2 | Kcnk2 | Mm.33304, Mm.387016 | 1.007 ± 0.035 |
| sodium channel, voltage-gated, type X, alpha polypeptide | Scn10a | Mm.247042 | 1.001 ± 0.033 |
| potassium channel tetramerisation domain containing 4 | Kctd4 | Mm.390712 | 1.011 ± 0.047 |
| leucine-rich repeats and transmembrane domains 2 | Lrtm2 | Mm.121498 | 1.012 ± 0.085 |
| Potassium channel tetramerisation domain containing 20 | Kctd20 | Mm.435 | 1.012 ± 0.014 |
| potassium inwardly-rectifying channel, subfamily J, member 3 | Kcnj3 | Mm.5127, Mm.406902 | 1.014 ± 0.015 |
| potassium voltage-gated channel, subfamily G, member 2 | Kcng2 | Mm.279568 | 1.015 ± 0.022 |
| chloride channel 4-2 | Clcn4-2 | Mm.297883 | 1.022 ± 0.019 |
| Amiloride-sensitive cation channel 1, neuronal (degenerin) | Accn1 | Mm.234998 | 1.023 ± 0.105 |
| potassium voltage-gated channel, subfamily H (eag-related), member 3 | Kcnh3 | Mm.374793 | 1.024 ± 0.042 |
| InaD-like (Drosophila) | Inadl | Mm.90218 | 1.026 ± 0.070 |
| Potassium voltage-gated channel, subfamily Q, member 5 | Kcnq5 | Mm.336519 | 1.029 ± 0.036 |
| ANKTM1 | Anktm1 | Mm.186329 | 1.030 ± 0.033 |
| potassium voltage-gated channel, subfamily H (eag-related), member 1 | Kcnh1 | Mm.4489 | 1.032 ± 0.048 |
| Kelch-like 28 (Drosophila) | Klhl28 | Mm.248678 | 1.036 ± 0.034 |
| transmembrane channel-like gene family 2 | Tmc2 | Mm.219546 | 1.037 ± 0.032 |
| potassium voltage-gated channel, subfamily H (eag-related), member 7 | Kcnh7 | Mm.242532 | 1.038 ± 0.026 |
| voltage dependent calcium channel gamma 5 subunit | Cacng5 | Mm.87663 | 1.038 ± 0.026 |
| chloride channel, nucleotide-sensitive, 1A | Clns1a | Mm.21482 | 1.038 ± 0.009 |
| potassium channel, subfamily U, member 1 | Kcnu1 | Mm.289679 | 1.041 ± 0.015 |
| potassium inwardly-rectifying channel, subfamily J, member 5 | Kcnj5 | Mm.69472 | 1.044 ± 0.019 |
| voltage-dependent anion channel 1 | Vdac1 | Mm.3555 | 1.044 ± 0.019 |
| coenzyme Q2 homolog, prenyltransferase (yeast) | Coq2 | Mm.260661 | 1.044 ± 0.049 |
| potassium channel tetramerisation domain containing 10 | Kctd10 | Mm.238285 | 1.046 ± 0.020 |
| transient receptor potential cation channel, subfamily C, member 3 | Trpc3 | Mm.74363 | 1.047 ± 0.018 |
| chloride intracellular channel 1 | Clic1 | Mm.29524 | 1.048 ± 0.011 |
| RIKEN cDNA 8230402K04 gene (8230402K04Rik), no gene name on unigene | not listed | Mm.124498 | 1.052 ± 0.028 |
| calcium channel, voltage-dependent, beta 3 subunit | Cacnb3 | Mm.3544 | 1.052 ± 0.028 |
| amiloride-sensitive cation channel 3 | Accn3 | Mm.299636 | 1.052 ± 0.047 |
| potassium channel tetramerisation domain containing 2 | Kctd2 | Mm.276299 | 1.053 ± 0.078 |
| potassium inwardly-rectifying channel, subfamily J, member 12 | Kcnj12 | Mm.4970 | 1.053 ± 0.065 |
| potassium voltage gated channel, Shaw-related subfamily, member 4 | Kcnc4 | Mm.101976 | 1.056 ± 0.037 |
| sodium channel modifier 1 | Scnm1 | Mm.182944 | 1.057 ± 0.026 |
| chloride channel 6 | Clcn6 | Mm.89987 | 1.059 ± 0.046 |
| potassium channel tetramerisation domain containing 3 | Kctd3 | Mm.209880, Mm.406821, Mm.432948 | 1.063 ± 0.014 |
| transient receptor potential cation channel, subfamily V, member 2 | Trpv2 | Mm.288064 | 1.064 ± 0.039 |
| sodium channel and clathrin linker 1 | Sclt1 | Mm.331001 | 1.065 ± 0.046 |
| potassium channel tetramerisation domain containing 9 | Kctd9 | Mm.6720 | 1.066 ± 0.018 |
| potassium voltage-gated channel, shaker-related subfamily, member 3 | Kcna3 | Mm.30640 | 1.068 ± 0.057 |
| sodium channel, voltage-gated, type II, beta | Scn2b | Mm.229373 | 1.070 ± 0.058 |
| glutamate receptor, ionotropic, AMPA3 (alpha 3) | Gria3 | Mm.327681 | 1.071 ± 0.005 |

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| cation channel, sperm associated 2 | Catsper2 | Mm.384318 | 1.071 ± 0.018 |
| solute carrier family 8 (sodium/calcium exchanger), member 3 | Slc8a3 | Mm.149463 | 1.071 ± 0.021 |
| calcium channel, voltage-dependent, N type, alpha 1B subunit | Cacna1b | Mm.4424 | 1.075 ± 0.032 |
| transient receptor potential cation channel, subfamily C, member 1 | Trpc1 | Mm.149633 | 1.076 ± 0.021 |
| potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 | Kcnn3 | Mm.120250 | 1.082 ± 0.024 |
| potassium voltage gated channel, Shab-related subfamily, member 2 | Kcnb2 | Mm.156081 | 1.085 ± 0.032 |
| transmembrane channel-like gene family 4 | Tmc4 | Mm.360398 | 1.086 ± 0.072 |
| potassium voltage gated channel, Shaw-related subfamily, member 1 | Kcnc1 | Mm.249386 | 1.089 ± 0.061 |
| calcium channel, voltage-dependent, alpha 1F subunit | Cacna1f | Mm.289647 | 1.094 ± 0.013 |
| proteasome maturation protein | Pomp | Mm.332855 | 1.100 ± 0.029 |
| chloride intracellular channel 4 (mitochondrial) | Clic4 | Mm.257765, Mm.430849 | 1.101 ± 0.033 |
| potassium channel, subfamily V, member 1 | Kcnv1 | Mm.300079 | 1.106 ± 0.056 |
| sodium channel, voltage-gated, type VIII, alpha polypeptide | Scn8a | Mm.385012 | 1.107 ± 0.074 |
| calcium channel, voltage-dependent, P/Q type, alpha 1A subunit | Cacna1a | Mm.334658 | 1.111 ± 0.338 |
| potassium inwardly-rectifying channel, subfamily J, member 10 | Kcnj10 | Mm.254563 | 1.111 ± 0.080 |
| purinergic receptor P2X, ligand-gated ion channel, 2 | P2rx2 | Mm.55948 | 1.113 ± 0.019 |
| ATPase, Na+/K+ transporting, beta 1 polypeptide | Atp1b1 | Mm.4550 | 1.113 ± 0.011 |
| potassium channel tetramerisation domain containing 12 | Kctd12 | Mm.246466 | 1.114 ± 0.026 |
| tangerin (LOC114601), EH domain binding protein 1-like 1 | Ehbp111 | Mm.210447 | 1.115 ± 0.033 |
| potassium channel tetramerisation domain containing 11 | Kctd11 | Mm.239498 | 1.117 ± 0.020 |
| asparagine-linked glycosylation 10 homolog B (yeast, alpha-1,2-glucosyltransferase) | Alg10b | Mm.17853 | 1.117 ± 0.041 |
| potassium voltage-gated channel, shaker-related subfamily, member 1 | Kcna1 | Mm.40424 | 1.118 ± 0.034 |
| gap junction membrane channel protein alpha 8 | Gja8 | Mm.258183 | 1.118 ± 0.007 |
| potassium channel tetramerisation domain containing 12b | Kctd12b | Mm.271572, Mm.433607 | 1.123 ± 0.017 |
| calcium channel, voltage-dependent, gamma subunit 6 | Cacng6 | Mm.424047 | 1.124 ± 0.006 |
| potassium channel modulatory factor 1 | Kcmf1 | Mm.29194 | 1.125 ± 0.023 |
| calcium channel, voltage-dependent, alpha2/delta subunit 1 | Cacna2d1 | Mm.159842, Mm.173392 | 1.129 ± 0.079 |
| potassium voltage-gated channel, shaker-related subfamily, beta member 1 | Kcnab1 | Mm.316402 | 1.129 ± 0.114 |
| voltage-dependent anion channel 2 | Vdac2 | Mm.262327 | 1.134 ± 0.058 |
| topoisomerase (DNA) II alpha | Top2a | Mm.4237 | 1.135 ± 0.013 |
| potassium inwardly-rectifying channel, subfamily J, member 8 | Kcnj8 | Mm.1482 | 1.139 ± 0.042 |
| calcium channel, voltage-dependent, gamma subunit 8 | Cacng8 | Mm.357990 | 1.143 ± 0.098 |
| potassium channel tetramerisation domain containing 13 | Kctd13 | Mm.245897 | 1.143 ± 0.038 |
| potassium voltage-gated channel, Isk-related family, member 1-like | Kcne1l | Mm.58507, Mm.299425 | 1.155 ± 0.026 |
| gap junction membrane channel protein alpha 4 | Gja4 | Mm.24615 | 1.156 ± 0.041 |
| potassium large conductance calcium-activated channel, subfamily M, beta member 1 | Kcnmb1 | Mm.6206 | 1.165 ± 0.052 |
| voltage-dependent anion channel 3 | Vdac3 | Mm.227704, Mm.426265 | 1.170 ± 0.054 |
| epidermodysplasia verruciformis 2 | Ever2 | Mm.116675 | 1.171 ± 0.038 |
| FXYD domain-containing ion transport regulator 5 | Fxyd5 | Mm.1870 | 1.173 ± 0.013 |
| Mid-1-related chloride channel 1 | Mclc | Mm.214545 | 1.178 ± 0.023 |
| sodium channel, nonvoltage-gated, type I, alpha polypeptide | Scnn1a | Mm.144114 | 1.186 ± 0.010 |
| calcium channel, voltage-dependent, gamma subunit 2 | Cacng2 | Mm.277338, Mm.390811, Mm.400802 | 1.193 ± 0.050 |
| Potassium inwardly-rectifying channel, subfamily K, member 6 | Kcnk6 | Mm.24877 | 1.195 ± 0.033 |
| gap junction membrane channel protein beta 1 | Gjb1 | Mm.21198 | 1.197 ± 0.189 |
| purinergic receptor P2X, ligand-gated ion channel, 5 | P2rx5 | Mm.156893 | 1.206 ± 0.053 |
| potassium voltage-gated channel, Shal-related family, member 2 | Kcnd2 | Mm.320691 | 1.217 ± 0.039 |

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| TWIK-related individual K+ channel | Trik | Mm.329947 | 1.229 ± 0.056 |
| glutamate receptor, ionotropic, AMPA4 (alpha 4) | Gria4 | Mm.209263 | 1.242 ± 0.056 |
| BTB (POZ) domain containing 5 | Btbd5 | Mm.248678 | 1.261 ± 0.107 |
| calcium channel, voltage-dependent, beta 1 subunit, transcript variant 1 | Cacnb1 | Mm.41252, Mm.391757 | 1.262 ± 0.025 |
| calcium channel mRNA, Two pore channel 1 | Tpcn1 | Mm.114054 | 1.359 ± 0.118 |
| potassium voltage-gated channel, subfamily Q, member 2 | Kcnq2 | Mm.40615 | 1.361 ± 0.100 |
| protein distantly related to to the gamma subunit family | Pr1 | Mm.24750 | 1.471 ± 0.032 |
| potassium voltage-gated channel, Isk-related subfamily, gene 3 | Kcne3 | Mm.282386 | 1.488 ± 0.039 |
| transmembrane channel-like gene family 7 | Tmc7 | Mm.69380 | 1.563 ± 0.071 |
| sodium channel, voltage-gated, type I, beta polypeptide | Scn1b | Mm.1418 | 1.618 ± 0.033 |
| transient receptor potential cation channel, subfamily C, member 7 | Trpc7 | Mm.62409 | 1.628 ± 0.146 |

**Figure S1. Frozen sections of R26R/Tg-Cre-ER$^{TM}$ stained for β-galactosidase including aortic valve (A) and pulmonary valve (B).** Bars = 500 um. AoV, aortic valve; PV, pulmonary valve.; LA, left atrium; LV, left ventricle, RV, right ventricle.

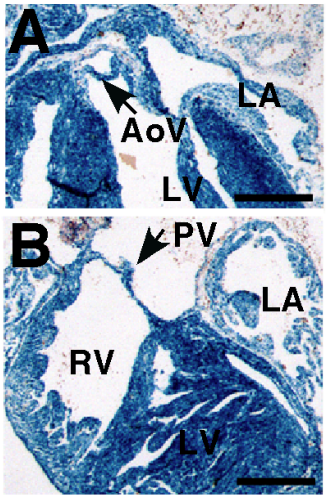

**Figure S2. Conduction defects (A) and increased heart weight (B) in the mice survived at PD28 in Nkx2-5 knockout (n=4) compared to control (n=5).** Either lead I (I) or lead II (II) ECG recording of each mouse is shown.

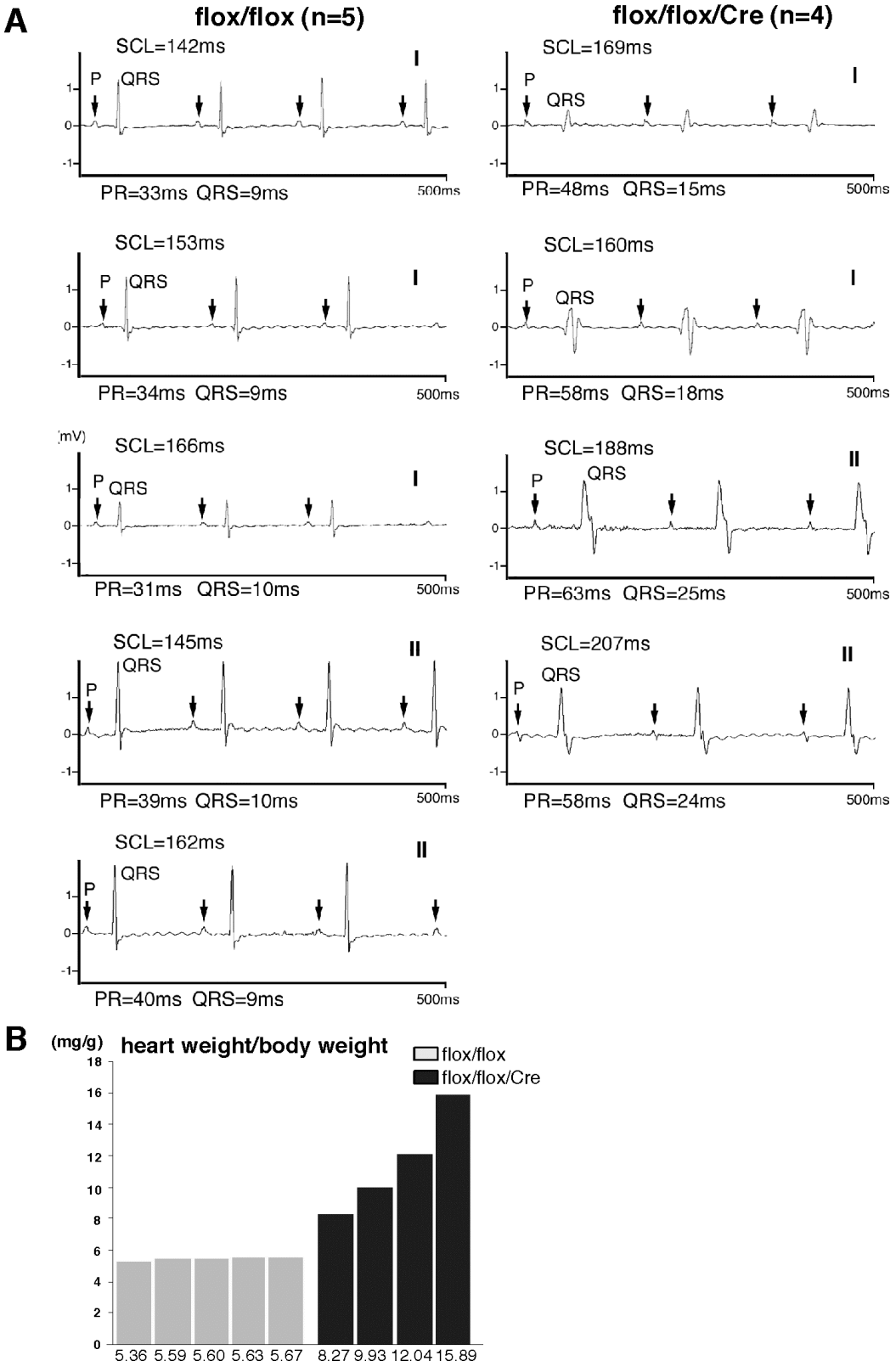

**Figure S3. Specificity of $Na_v1.5$ staining.** Anti-$Na_v1.5$(□) antibody (alomone labs, ASC-005) was utilized for immunohistochemistry in control flox/flox hearts in the absence (left) and presence (right) of pre-adsorption with antigen-peptide (1 □g peptide /1 □g antibody). Staining was performed side by side.

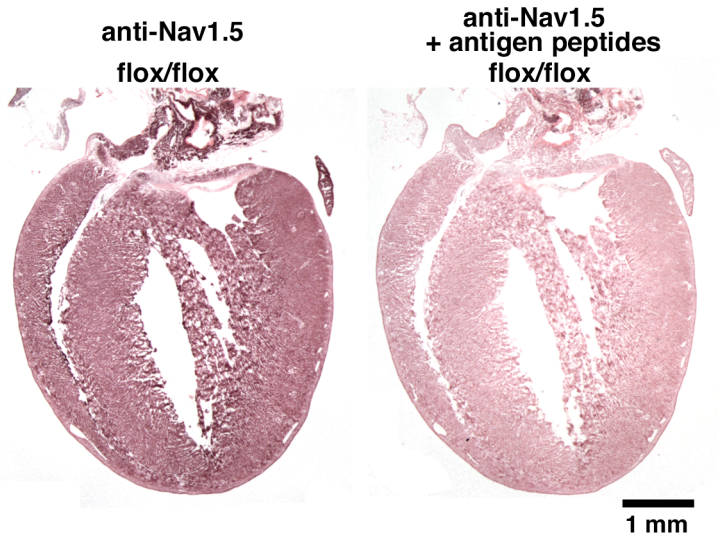


**Figure S4. Immunostaining of $Na_v1.5$(□) in two additional flox/flox/Cre mouse hearts at PD12.** Reduced staining of $Na_v1.5$(□) in ventricles, but preserved staining in atria, His bundles and inner layer of ventricles (likely Purkinje fibers) was noted.

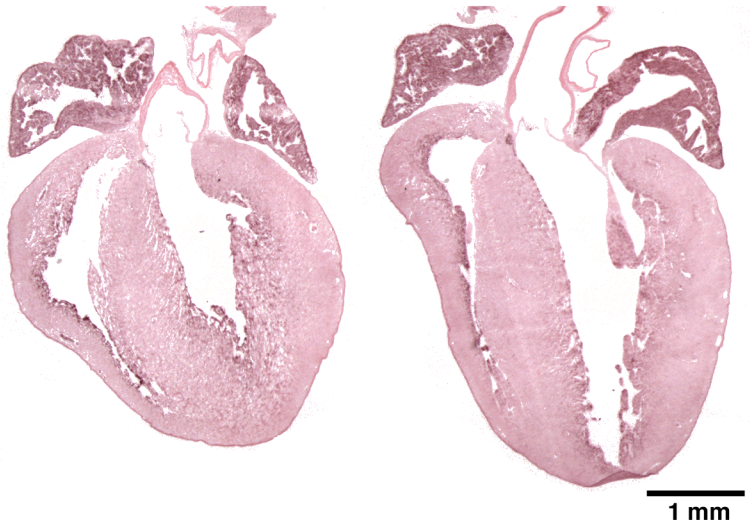

**Figure S5. Western blotting demonstrates similar levels of $Na_v1.5$(□) protein in atria of flox/flox and flox/flox/Cre mice (lanes 1 vs.2).**