SUPPLEMENTAL MATERIAL

Expanded Materials and Methods

Plasmid Construction

The rat SkM1 insert was released from pμI-2-SkM1, kindly provided by Dr. Gail Mandel (SUNY, Stony Brook, NY) and subcloned into a mammalian expression vector pIRES2-EGFP (BD Bioscience Clontech, Mountain View, CA) at the EcoR I site. Human SCN5A-pcDNA3.1 was a kind gift from Dr. Robert Kass (Columbia University, New York, NY). The insert was excised using Hind III and Xba I restriction enzymes, blunt ended, and subcloned into the Sma I site of pIRES2-EGFP.

Cell Isolation and Culture

Canine mesencheymal stem cells were isolated by Ficoll-Paque Plus density gradient centrifugation from aspirated bone marrow (Figure S1). Primary cultures of cMSC were maintained at 37℃ in 5% CO2/95% air with an initial medium for 48h. Medium was then changed every 3-4d. Cell colonies with spindle-like morphology were transferred 7d after initial plating. After confluence cells were harvested with 0.25% trypsin-EDTA, and replated. Isolated cells were characterized at passages 2-4 by flow cytometric analysis of specific surface antigens with fluorescein isothiocyanate- (FITC) conjugated rat anti-canine CD44, FITC-conjugated rat anti- canine CD45 unconjugated rat anti-canine CD90, and phycoerythrin-(PE) conjugated mouse anti-canine CD34. A large majority of the cells were CD44 (99.61%) and CD90 (93%) positive – and 98% were CD34 and CD45 negative; suggesting a significant majority were MSCs (Figure 2S).

To further validate the MSC properties of the isolated cells we subjected subsets of the cells to osteogenic, apdipogenic and chondrogenic differentiation protocols. For

adipogenic and osteogenic differentiation, the cells were plated in 6-well or 12-well plates. Adipogenic and osteogenic induction was initiated using designated kits from Lonza. For adipogenesis, three to five cycles of the following media changes were performed: 2-3 days of exposure to adipogenic induction medium followed by 2-3 days of exposure to maintenance medium. Osteogenic induction was carried out by feeding the cells with osteogenic induction medium every 3-4 days for 2-3 weeks. Chondrogenic induction was performed by pelleting 2.5×10^5 cells in chondrogenic induction medium containing TGF-β3. Complete media changes were performed every 2-3 days for 3-4 weeks. At the end of the induction protocols, the cells were rinsed with PBS and fixed with 10% formalin. Adipogenesis was assayed using Oil Red O staining. Osteogenesis was assayed by staining for calcium deposition using Alizarin Red staining. Chondrogenic pellets were embedded in cryogenic cutting medium, sectioned for histology, and glycosaminoglycans were stained using Safranin O. Figure S3 illustrates the osteogenic, apdipogenic and chondrogenic potential of the isolated cells.

Transfection

cMSCs were transfected with pIRES2-EGFP-SkM1 or -SCN5A construct by electroporation using Nucleofector technology (Amaxa Lonza, Gaithersburg, MD) as directed and were incubated at normal culture conditions. Expression of EGFP and Na^+ currents was examined 24-48h after transfection. 30-45% of the cells were GFP positive. Na^+ currents were measured in GFP-positive cells perfused with 15 mM Na^+ Tyrode's solution. cMSC/SkM1 and cMSC/SCN5A cell capacitances were 57.63 ± 6.74 pF and 56.38 ± 6.93 pF, respectively (n=8/group).

Patch clamp studies

Whole cell patch clamp with a signal amplifier (Model Axopatch-1B, Axon Instruments Inc.) was used to measure single cell membrane current. Voltage and current signals were digitized (Model DIGIDATA 1320A, Axon Instruments) and transferred to a personal computer. Data acquisition and analysis were performed using CLAMPEX 9.2 and CLAMFIT 9.2 software (Axon instruments), respectively. Normal Tyrode's solution contained (mM): NaCl 137.7, KCl 5.4, NaOH 2.3, $CaCl_2$ 1.8, $MgCl_2$ 1, Glucose 10, and HEPES 10 (pH adjusted to 7.4 with NaOH). Low Na^+ Tyrode's solution contained (mM): NaCl 15, TEACl 122.7, KCl 5.4, NaOH 2.3, $CaCl_2$ 1.8, $MgCl_2$ 1, Glucose 10, and HEPES 10 (pH adjusted to 7.4 with NaOH). Electrodes with resistances =3-4 MΩ were made from capillaries with a P-87 Flaming/Brown micropipette puller (Sutter Instrument Company, Novato, CA) and filled with (mM): KCl 50, K-aspartic acid 80, $MgCl_2$ 1, EGTA 10, HEPES 10 and Na_2-ATP (pH adjusted to 7.2 with KOH). The liquid junction potential (~8 mV between bath and electrode solutions) was not corrected because exchange between pipette and cell are never complete. [1]

Generation of a cardiac Syncytium

Neonatal Sprague-Dawley rats were sacrificed and ventricular myocytes were isolated by an approved Stony Brook University IACUC protocol as previously described.[2] Ventricles were excised and washed free of blood, tissue cut into small pieces and enzymatically digested with trypsin at 4℃ (1mg/ml, USB, Cleveland, OH), and, the next morning, with collagenase at 37℃ (1mg/ml, Worthington, Lakewood, NJ). Cardiac fibroblasts were removed by 90 min preplating. Isolated ventricular myocytes were re-plated at 4×10^5 cells/cm^2 for the control group and 3.5×10^5 cells/cm^2 for the coculture groups at a 20:1 ratio with cMSC onto grooved fibronectin-coated polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) scaffolds in M199 medium (GIBCO Invitrogen) supplemented with 10% fetal bovine serum (GIBCO Invitrogen) for

2d and then reduced to 2%. Cultures were maintained in an incubator at 37℃ with 5% CO_2 for 4-5d before functional measurements.

Microscopic Dynamic Functional Measurements and Analysis

All scaffolds were washed and equilibrated at room temperature in normal Tyrode's solution. Samples were then stained with Fluo-4 AM (Invitrogen, Carlsbad, CA) for 20 min for tracking Ca^{2+} waves. After washing with Tyrode's, scaffolds were removed from the surface so that only the cells in the grooves remained, forming 5 linear cultures. A 2-D optical mapping system[3] as in the companion paper[4] was used to measure impulse propagation. To vary the degree of membrane depolarization, we used 5.4 and 10.4 mM K^+ Tyrode's solutions.

Induction of VT/VF

Pacing threshold was determined by incrementally increasing the current until capture. Ventricular pacing was performed at 2X pacing threshold. Extrastimulus pacing with a programmable stimulator (Bloom Associates, Reading, Pa) was performed sequentially in the high paraseptal region (PS), infarct lateral EBZ/injection region and within the infarct. Pacing trains began with 10 stimuli at a cycle length of 350-400 ms. S2 was initiated at 250 ms, and S1-S2 was decreased in 10-ms steps until loss of capture. For S3 pacing, S1-S2 was set at the shortest interval with reliable S2 capture (equivalent to the effective refractory period; ERP). S3 was initiated at a coupling interval of 100 ms and increased in 10-ms steps until S3 capture occurred. If VT was ≥60 seconds or when the protocol was finished, the heart was removed and prepared for microelectrode study, histology, and infarct sizing.

Microelectrode Methods

Hearts were removed and immersed in Tyrode's solution[5] equilibrated with 95% O2/5% CO2. Epicardial strips (≈10X5 X 0.5 to 1 mm) were filleted parallel to the left ventricular free wall surface from non-injected EBZ sites and EBZ sites injected with blank cMSC or cMSC/SkM1. Preparations were pinned epicardial surface up to the bottom of a 4-mL tissue bath and superfused (36°C, pH 7.35±0.05) at 12 mL/min. Superfusate $[K^+]$ was varied from 4-7 mmol/L to permit V_{max} measurement at various membrane potentials. Preparations were paced at a cycle length of 500 ms, and AP recorded at 30–50 sites/preparation after 3h equilibration to reach steady state.[5]

Infarct Sizing

After tissues were removed for microelectrode study and histology, the heart was cooled to 4°C and cut into 1-cm-thick transverse slices from apex to base. Slices were incubated x20 min in 1% tetrazolium red (pH, 7.4 buffer at 37°C), immersed in 10% formalin x15 min, and pressed between 2 glass plates to obtain uniform 1-cm thickness. Apical sides of slices were photographed, and a digital image planimetered (Image J Analysis 1.40g, National Institutes of Health, Bethesda) to determine overall infarct size. Volume of infarcted myocardium was calculated by multiplying planimetered areas by slice thickness and expressed as % total left ventricular volume.

Histology and Immunochemistry

Tissue blocks were snap-frozen in liquid nitrogen; 5 μm serial sections were cut with a cryostat (Microm HM505E) and air-dried. Sections were washed in PBS, blocked x20 min with 10% goat serum, and incubated overnight at 4°C with anti-SkM1 antibody (1:200, Sigma-Aldrich, St Louis, Mo) alone or together with Cx43 antibody (1:500,Invitrogen, Breda, The Netherlands). Antibody bound to target antigen was detected by incubating sections x2h with goat anti-mouse IgG

labeled with Cy3 (red fluorescence for SkM1) and goat anti-rabbit IgG labeled with Alexa 488 (green fluorescence for Cx43), together with detection of GFP with a Nikon E800 fluorescence microscope.

Western Blotting

Tissue samples were sonicated in lysate buffer, which contains 1X PBS, 1% Triton X-100, 0.5% NaDoc, 0.1% Tween-20 and protease inhibitor tablet (Roche), for 45 second and incubated on ice for 30 min. After centrifugation at 3000 rpm for 10 minutes to remove connective tissue and unbroken cells, the supernatant were used as the whole cell lysate. Samples were separated on a 4-20% tris-glycine gradient gel (invitrogen) and transferred to PVDF membrane (Biorad). After blocking with 5% milk for an hour at room temperature membranes were incubated with anti-Nav1.4 mouse monoclonal antibody (Sigma-Aldrich, 1:500) or anti-GAPDH rabbit polyclonal antibody (Fitzgerald Industries International, 1:5000) in 5% milk overnight at 4° C. After washing, membranes were incubated with secondary antibody for an hour at room temperature (GE Healthcare), followed by enhanced chemiluminescence processing (Amersham Pharmacia Biotech). In Figure 6B, the sample neonatal rat ventricular myocytes (NRVMs) transduced with a SkM1 overexpressing adenovirus (Ad-SkM1)[6] was used as a positive control - loaded to the gel with 1 ug/lane (as compared to 75 ug/lane for the other samples) - and is not included for quantitative comparison. The results for the cMSC/SkM1-treated animal is typical for a total of 4 animals tested.

Legends

Figure S1. Canine mesenchymal stem cell (cMSC) isolated by Ficoll-Paque Plus density gradients centrifugation. A, Cells isolated from the density interface of 1.073g/ml attached and grew as symmetric spindle-likes morphology cell colonies in about 7-10 days after initial

planting. B-C, Cells obtained from densities higher than 1.073g/ml showed different cell morphology and did not develop into colonies over 7-10 days.

Figure S2. Flow cytometric identification of Ficoll-Paque Plus density gradient isolated cMSC. A Typical flow cytometry histograms indicating nearly all cells are negative for CD34 (2.01%; upper panel) and CD45 (2.18%; lower panel). B, Typical flow cytometry histograms indicating a majority of cells are positive for CD90 (93.45%; upper panel) and nearly all cells are positive for CD44 (99.61%; lower panel).

Figure S3. Canine MSCs differentiation. A-B, Osteogenic differentiation. B, Red-brown staining indicates Ca^{2+} deposition based on alkaline phosphatase - suggesting osteogenic differentiation in induced cultures. A, Non-induced cultures did not show Ca^{2+} depositions. C-D, Adipogenic differentiation. D, Positive Oil red-O staining indicates adipogenic differentiation in induced cells. C, Non induced cells did not show positive staining for Oil red-O. E-F, Chondrogenic differentiation. F, Cell pellets of induced cells showed positive safranin O staining – indicative for presence of proteoglycans and therefor suggesting cartilage formation. E, Pellets of non-induced cells do not show safranin O positivity. Cytoplasm stains green and nuclei stain black.

Figure S4. Recovery of SkM1 current (A) and SCN5A current (B) in cMSCs. Cells were held over a range of holding potentials and double-pulsed to 0 mV. Each pulse had a 10 msec duration, with an increasing time interval between the two pulses. Data were normalized to the current amplitude of the first pulse (n = 8 for each holding potential) and curve fit the equation $f = 1- \exp(-t/\tau)$. (C) Comparison of recovery time constant τ between SkM1 and SCN5A at different holding potentials.

References

1. Mathias RT, Cohen IS, Oliva C. Limitations of the whole cell patch clamp technique in the control of intracellular concentrations. *Biophys J* 1990;58:759-770.

2. Bien H, Yin L, Entcheva E. Calcium instabilities in mammalian cardiomyocyte networks. *Biophys J* 2006;90:2628-2640.

3. Entcheva E, Bien H. Macroscopic optical mapping of excitation in cardiac cell networks with ultra-high spatiotemporal resolution. *Prog Biophys Mol Biol* 2006;92:232-257.

4. Lu J, Wang HZ, Jia Z, Zuckerman J, Lu Z, Guo Y, Boink GJJ, Brink PR, Robinson RB, Entcheva E, Cohen IS. Improving cardiac conduction with a skeletal muscle sodium channel by gene and cell therapy. *J Cardiovasc Pharmacol.* 2012: Epub ahead of print.

5. Sosunov EA, Anyukhovsky EP, Rosen MR. Effects of quinidine on repolarization in canine epicardium, midmyocardium, and endocardium: I. In vitro study. *Circulation* 1997;96:4011-4018.

6. Protas L, Dun W, Jia Z, Lu J, Bucchi A, Kumari S, Chen M, Cohen IS, Rosen MR, Entcheva E, Robinson RB. Expression of skeletal but not cardiac Na+ channel isoform preserves normal conduction in a depolarized cardiac syncytium. *Cardiovasc Res.* 2009;81:528-535.

Figures:

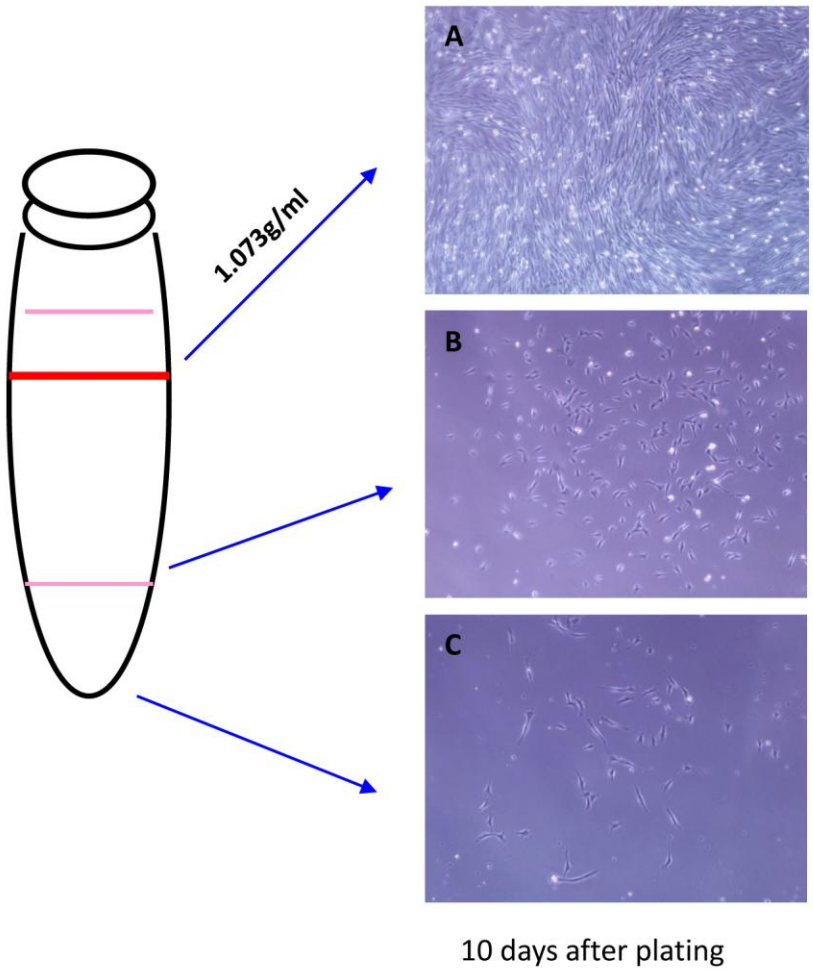
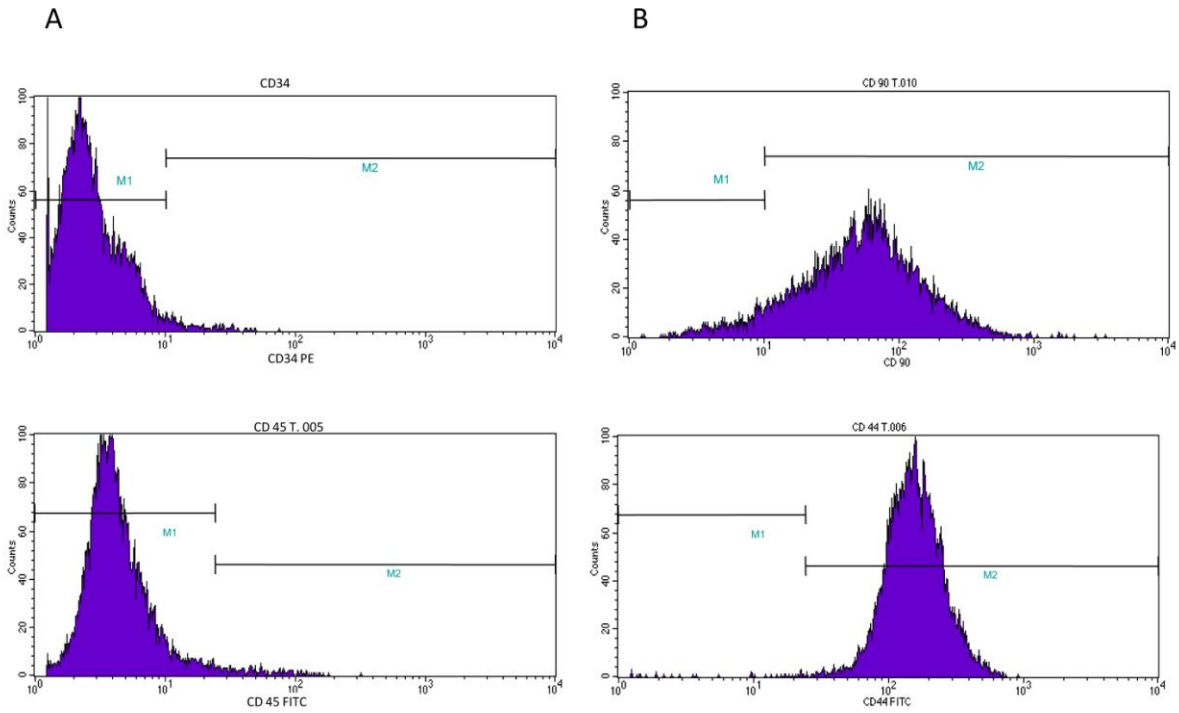


Figure S1


A
B
CD34
M1
M2
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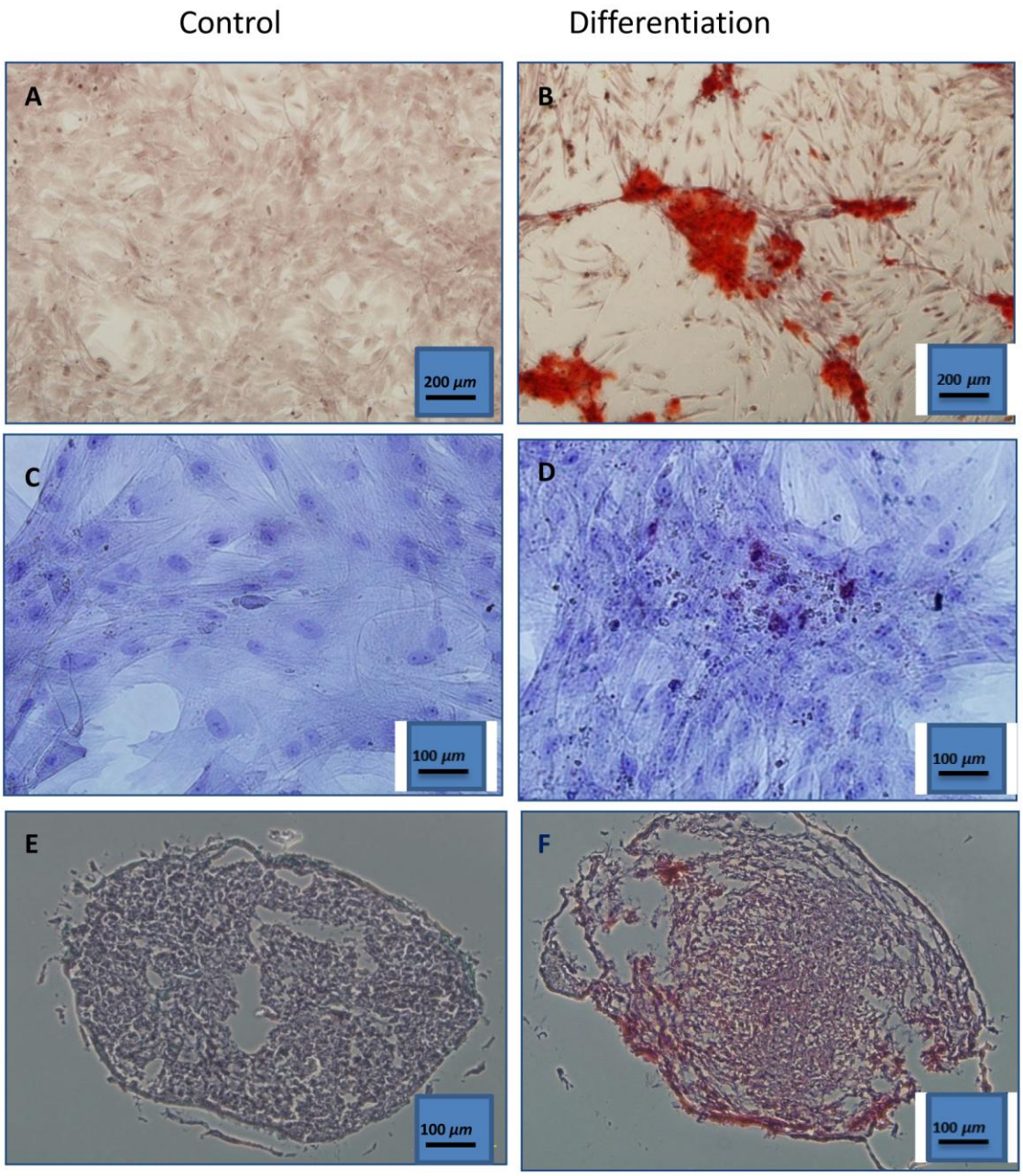
CD44 FITC

Figure S2


Control
Differentiation
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B
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C
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D
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E
100 µm
F
100 µm

Figure S3

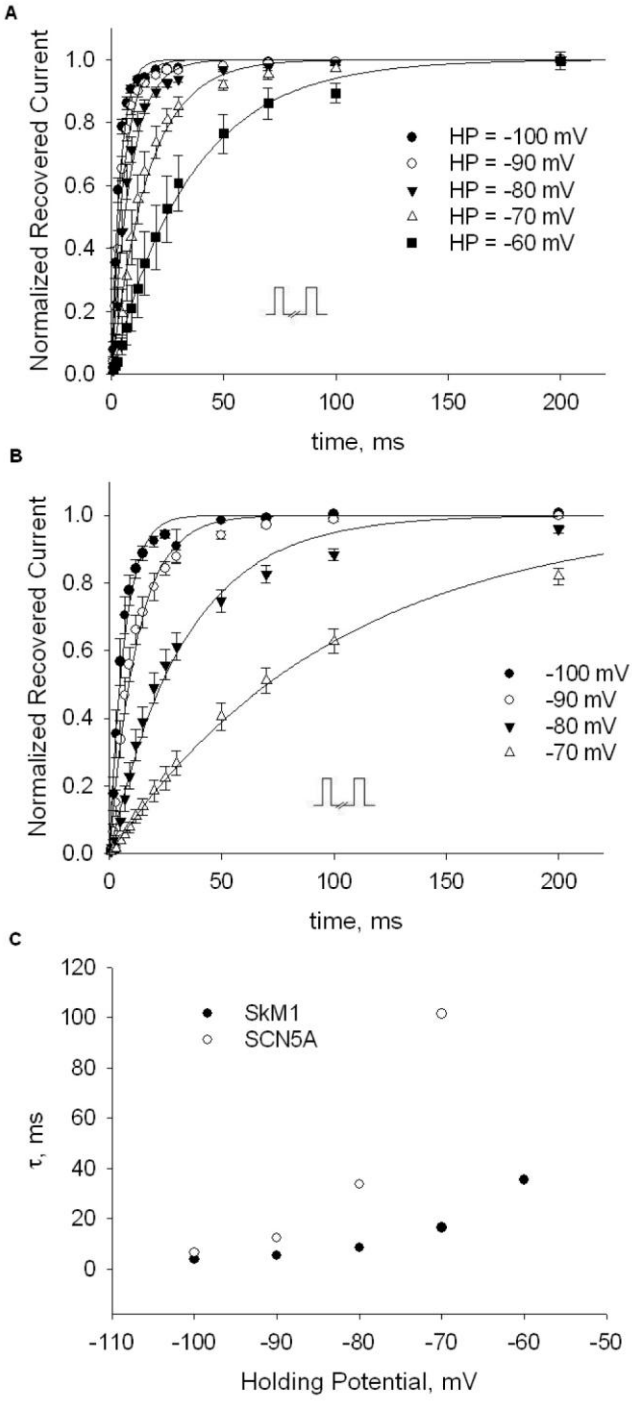
A
Normalized Recovered Current
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
time, ms
HP = -100 mV
HP = -90 mV
HP = -80 mV
HP = -70 mV
HP = -60 mV
B
Normalized Recovered Current
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
time, ms
-100 mV
-90 mV
-80 mV
-70 mV
C
τ, ms
120
100
80
60
40
20
0
-110
-100
-90
-80
-70
-60
-50
Holding Potential, mV
SkM1
SCN5A

Figure S4