Supplement Material

Expanded Methods

Identification of potential regulatory regions

To identify CNS elements, we compared the human *SCN5A* locus with its mouse ortholog using the VISTA Genome Browser (http://pipeline.lbl.gov/cgi-bin/gateway2).[1,2] Each of 92 CNS elements identified was then PCR amplified and assayed for activity as described below. Those showing >5-fold increase in reporter activity in luciferase assays were then analyzed for potential muscle-specific transcriptional regulatory modules using the M-SCAN algorithm (http://www.cisreg.ca/cgi-bin/mscan/MSCAN).[3,4] For identification of potential repressive transcription factors in CNS28 we used rVista (http://rvista.dcode.org/) to compare human and mouse sequences for conserved transcription factor binding sites.[5,6]

Generation of reporter constructs

Three sets of reporter constructs were made. Initial screening experiments examined luciferase reporter activity of CNS constructs in the pGL3-Promoter vector (Promega). Subsequently, we conducted a series of experiments examining the effect of CNS28 on the activity of the *SCN5A* core promoter and CNS28 truncation constructs with the previously identified alternate promoter.[7] Each of the 92 CNS identified in the initial VISTA scan was PCR-amplified thus generating a series of luciferase fusion constructs (pGL3-CNS1 through pGL3-CNS92). The PCR reactions used forward primers containing an *NheI* recognition sequence at the 5' end and reverse primers containing an *XhoI* recognition sequence (See Supplemental Table 1). PCR products were digested with *NheI* and *XhoI*, and the fragments were subcloned into the pGL3-Promoter vector.

The starting point for generating CNS28/SCN5A promoter constructs, was the pGL3-Basic vector containing the full length *SCN5A* promoter we previously generated.[8] CNS28 was amplified from human genomic DNA obtained from Vanderbilt University's Polymorphism Discovery Core, using modified versions of the CNS28 primers, F_*SalI* and R_*BamHI* (Table 1), and the Expand High Fidelity Kit (Roche) following the protocol provided. CNS28 was inserted into the vector using the *SalI/BamHI* sites downstream of the luciferase cDNA. All constructs were verified by direct sequencing.

Experiments were also conducted to analyze CNS28 truncation constructs. Human and mouse CNS28 sequences were compared and scanned for conserved transcription factors using rVista 2.0.[5,6] Five fragments were amplified based on the location of regulatory elements from the rVista analysis. DC1-F, DC2-F, and DC3-F (Table 1) remove the first 50, 150, and 280 base pairs from CNS28 respectively. The universal reverse primer, DC-R, as well as DC0-F and DC4-F (Table 1) amplify the alternate promoter previously identified,[7] with and without the whole CNS28 fragment respectively. PCR fragments were amplified using the Expand Long kit (Roche) with CNS28$^{-/-}$ mouse genomic DNA as the template. The cycling conditions used were an initial denaturing step of 94°C for 2' followed by 35 cycles of 94°C for 20", 65°C for 30", and 72°C for 2' and a final 7' extension at 72°C. PCR fragments were digested with *KpnI/BglII* and inserted into the pGL3-Basic vector (Promega). All constructs were verified by direct sequencing.

Mutagenesis

Mutagenesis on DC3 was performed using the QuickChange II XL kit (Stratagene) using the primers listed in Table 1. The standard protocol provided with the kit was followed and the construct was verified by direct sequencing.

Reporter assays

Experiments were conducted as previously described in CHO cells[8,9] and in cardiomyocytes[8-10] isolated from 1-2 day old mice. The pGL3-CNS/luciferase fusion genes (125 ng DNA) were transfected into the neonatal mouse cardiac cells using FuGENE 6 (Roche), and into CHO cells using lipofectamine reagent (Invitrogen). The pGL3-Promoter plasmid was tested in each experiment and its activity level served as the no insertion control and its activity was designated as 100%. The SCN5A Promoter and SCN5A Promoter-CNS28/luciferase genes (500 ng DNA) were transfected into both cell lines using FuGENE 6 (Roche) with the construct containing CNS28 designated as 100% activity. The CNS28 truncation constructs containing the alternate mouse promoter (250 ng DNA) were transfected into both cell lines using FuGENE 6 (Roche). The construct containing the full CNS28 sequence was designated 100% activity. In each experiment, the pRL-TK plasmid (5-10 ng), encoding Renilla luciferase, was co-transfected to normalize for experimental variability caused by differences in cell viability or transfection efficiency. Luminescence was measured after 48 hr transfection by using Dual-Luciferase Reporter Assay System (Promega). The DC3 mutant transfection and luciferase assay were performed in an identical manner to the truncation constructs.

Generation of CNS28-KO mice

Mouse embryonic stem (ES) cells in which a region of the Scn5a gene was modified to allow RMCE to be used to easily generate mice containing allelic variant of human sodium channels under control of the Scn5a regulatory sequences.[11] Using this approach, we generated H/H mice in which the targeted region was replaced by the human SCN5A full-length cDNA. For the present experiments, we modified the original H exchange construct to delete bases 1720 to 2154 (that encompass the CNS28 region), and then performed RMCE as previously described to generate $CNS28^{+/-}$ animals. After removal of a hygromycin resistance cassette by breeding to a FlpE-expressing line, mice were then interbred to generate $CNS28^{-/-}$ animals.[11] Experiments began after three backcrosses using littermate H/H mice as controls. All experiments performed on mice were approved by the institutional animal care and use committee.

Quantitative Real Time RT-PCR

Total mRNA from adult mice atria or ventricles was isolated using the TRIzol method (Invitrogen) and cDNA was prepared using the Transcriptor First Strand cDNA Synthesis kit (Roche). Quantitative Real Time RT-PCR (qPCR) on *SCN5A* was performed with TaqMan probes targeting human *SCN5A* (Hs00165693_m1) using beta actin (Mm00607939_s1) as a reference gene. qPCR targeting mouse *Scn1b* was performed using TaqMan probe (Mm00441210_m1) with *Hprt1* (Mm00446968_m1) as the reference gene. The reactions were performed using the TaqMan Fast Universal PCR Master Mix in a 7900HT thermocycler (Applied Biosystems). Cycling conditions were an initial step of 15" at 95° C, followed by 40 cycles of 1" at 95° C and 10" at 60° C. Standard dilutions of plasmids containing the target genes were used to quantitate the amounts of mRNA present in our samples. Relative mRNA amounts were calculated comparing human SCN5A to mouse beta actin levels and were normalized to the levels in H/H mice. Analysis was performed using SDS 2.2.2 software (Applied Biosystems)

Western blotting

Western blot analysis of $Na_v1.5$ (SCN5A protein) and calnexin was performed using 100ug of whole heart protein extract. Blots were cut to separate the SCN5A and calnexin bands and were stained with anti-SCN5A antibody (Alomone ASC-005 1:250 dilution) or anti-calnexin antibody (Stressgen SPA860 1:2000 dilution) respectively. The secondary antibody was anti-rabbit IgG HRP-conjugate (Promega W401B 1:10,000 dilution). Films were scanned on a standard desktop scanner and band density was measured using ImageJ, freely available from the NIH. Relative protein levels were

calculated for each sample by comparing band densities for SCN5A and calnexin signals.

Electrocardiogram (ECG) recordings and lidocaine challenge

ECGs and drug challenges were recorded as previously described.[12] In brief, adult mice were anesthetized with isoflurane vapor adjusted to maintain the light anesthesia and a constant heart rate throughout the recording procedure. Electrodes were inserted subcutaneously in each limb and baseline recordings were obtained over 5 minutes. For lidocaine challenge, ECGs were recorded at baseline as described above and for an additional 20 minutes after intraperitoneal injection of lidocaine 40 mg/kg (Sigma). ECG signals were averaged over 10 second epochs and intervals were obtained from the signal averaged tracings using a custom signal averaging program using National Instruments LabView.

Isolation of mouse ventricular cardiomyocytes and sodium current recordings

Adult H/H and CNS28$^{-/-}$ mouse cardiac ventricular myocytes were isolated by a modified collagenase/protease method.[13] After intraperitoneal injection of 500 IU of heparin, adult mice were anesthetized using inhaled isoflurane/oxygen mixture, hearts excised, and their aortae rapidly cannulated and perfused with modified Tyrode's solution (MTS) for 3 min followed by MTS containing collagenase (Liberase Blendzyme-4, Roche, 0.04 mg/ml) for 5–7 min at a constant pressure of 80 mmHg and temperature of 34 ºC. The MTS contained (in mmol/l) NaCl 130, HEPES 10, glucose 10, KCl 5.4, $MgCl_2$ 1.2, NaH_2PO_4, 2,3-butanedione monoxime 10, pH of 7.2. The digested ventricles were minced in MTS containing 1 mg/ml bovine serum albumin and 0.2 mmol/l CaCl2 and triturated by gently pipetting. The resulting solution was strained and the myocytes allowed to sediment in MTS of increasingly higher Ca^{2+} concentrations (0.2, 0.5, and 1 mmol/l). This procedure routinely yielded 60–80% rod-shaped, Ca^{2+}-tolerant myocytes that were used for the electrophysiology studies.

To control INa, external sodium concentration was lowered to 5 mM, wide-tip electrodes with tip resistance <1 MΩ were used, and experiments were conducted at 18ºC. INa was recorded using whole-cell voltage clamp. The pipette-filling (intracellular) solution contained (in mmol/L): NaF 10, CsF 110, CsCl 20, EGTA 10, HEPES 10, with a pH of 7.4 adjusted with CsOH. The bath (extracellular) solution had (in mmol/L): NaCl 5, CsCl 5, TEA-Cl 135, CaCl2 0.1, MgCl2 1, HEPES 10, and glucose 10, with a pH of 7.4 adjusted by CsOH. To eliminate L- and T-type inward calcium currents, 0.5 µM nisoldipine and 200 µM NiCl were added to the bath solution. Data acquisition was carried out using an Axopatch 200B patch-clamp amplifier and pCLAMP version 9.2 software (MDS Inc., Mississauga, Ontario, Canada). Currents were filtered at 5 kHz (-3 dB, four-pole Bessel filter) and digitized using an analog-to-digital interface (Digidata 1322A, MDS Inc.). To minimize capacitive transients, capacitance, and series resistance were adjusted to 70–85%. The holding potential was -120 mV for all experiments, and details of the pulse protocols are presented schematically in the figures. INa densities in H/H and CNS28$^{-/-}$ mice were compared as pA/pF after normalization to cell sizes which were generated from the cell capacitance calculated by Membrane Test (OUT 0) in pClamp 9.2. Electrophysiologic data were analyzed using Clampfit version9.2 (Axon Instruments), and figures were prepared by using Origin 7.0 (OriginLab Corp., Northampton, MA, USA). Current-voltage relations for steady-state activation and inactivation were fit with the Boltzmann equation I/Imax = (1 + exp[(V - V1/2)/k])-1 to determine the membrane potential for half-maximal activation (V1/2-activation) and inactivation (V1/2-inactivation).[11]

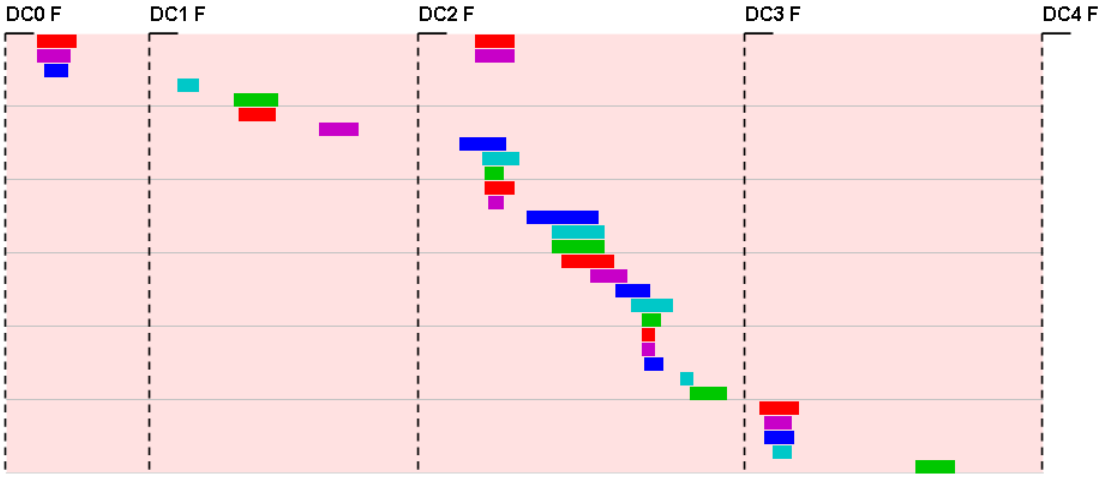
Echocardiogram

Transthoracic echocardiograms were performed on resting conscious mice at the Murine Cardiovascular Core, Vanderbilt University as previously described.[14] Signals

were acquired using a 15-MHz transducer (Sonos 5500 system, Agilent) and analyzed by a sonographer who was blind to the genotype.

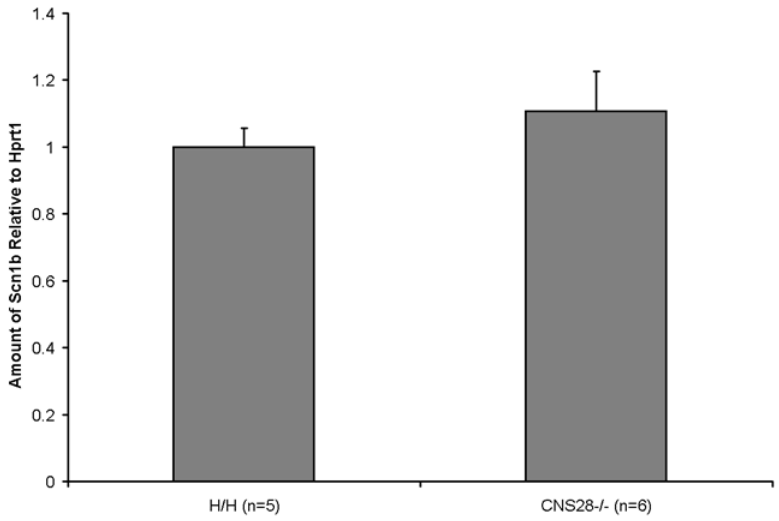
Data analysis

Results are presented as mean±SE, and statistical comparisons were made using the unpaired Student's t test. A value of $P<0.05$ was considered statistically significant.



Online Figure I

Selection of deletion construct primer locations. Using the rVista output of the human and mouse CNS28 alignments as a guide, locations for the forward primer of the deletion constructs were chosen within the gaps between clusters of predicted transcription factor binding sites.



Online Figure II

Relative amount of *Scn1b*. Using Real-Time PCR we analyzed amounts of *Scn1b* transcript relative to *Hprt1* levels. There was no significant difference between H/H mice and CNS28$^{-/-}$ mice.

Online Table I. Primer pairs used to amplify CNS (In Order of Position)

Forward Primer	Reverse Primer	Position
CNS-2F: GCGTGCTAGCCGAGTGGTTGATGTCTCCGCCCT	CNS-2R: AGATCTCGAGTACTGGAAGTCAATGCTTCGGG	5'-end
CNS-3F: GCGTGCTAGCCTACCTGGCAGAAATGGGTACAT	CNS-3R: AGATCTCGAGCAGACTTTACCCAT TCTACTGC	5'-end
CNS-6F: GCGTGCTAGCCTTCCAGGAGACATTTACAAGAC	CNS-6R: AGATCTCGAGCCTGGCTTCTTTCATTTAATAAGG	5'-end
CNS-7F: GCGTGCTAGCCTAACTTCTACAAATCAATGCAA	CNS-7R: AGATCTCGAGATAGATGTGTGGTGGTATTTCA	5'-end
CNS-8F: GCGTGCTAGCCCCCGTCATCTACATTAGGTAAT	CNS-8R: AGATCTCGAGCAAGTCAATCCTAATCCTGTTT	5'-end
CNS-9F: GCGTGCTAGCCCAGTGAACTCTGGCTCCAGAAA	CNS-9R: AGATCTCGAGAACTGGTATTTAAAGGGTCAGC	5'-end
CNS-10F: GCGTGCTAGCCACATAACAGCAAACTGATTCACATC	CNS-10R: AGATCTCGAGGTGTGCTTGCATTTTAAAGGAA	5'-end
CNS-78F GCGTGCTAGCCGTGGGTTGAAACGTTGACACT	CNS-78R AGATCTCGAGTGAGTGGGGTGTCTGGGGTACA	5'-end
CNS-79F GCGTGCTAGCCGGTATTTCTCCTAATGCTAAAA	CNS-79R AGATCTCGAGGATTGGCTAATTGTTTCTTAGC	5'-end
CNS-88F GCGTGCTAGCCGGGTGTCTGGTAGCCTTCCCAA	CNS-88R AGATCTCGAGAGCGATCCCTGCATCCTACGGG	5'-end
CNS-89F GCGTGCTAGCCGTGTACCATTGTGCAGATTTCT	CNS-89R AGATCTCGAGACACACACTGACACTGAGGAAG	5'-end
CNS-90F GCGTGCTAGCCCTGGGCCCTAGTGAGAACACT	CNS-90R AGATCTCGAGGTTCTGACATTGTGACCAGTGG	5'-end
CNS-1F: GCGTGCTAGCCGCTGGAGGCGGAGATTAAGAGT	CNS-1R: AGATCTCGAGAAGACTTCTACCTGCTTTGGGG	intron1
CNS-4F: GCGTGCTAGCCTCTGTGCTGAAAAGAGCCTCAT	CNS-4R: AGATCTCGAGGCTCAGTTCTAGGCTTATTTGC	intron1
CNS-5F: GCGTGCTAGCCTTTGCAGCGACAGCCTCACCAC	CNS-5R: AGATCTCGAGCCATAGACCCCAAGGGATCCTT	intron1
CNS-11F: GCGTGCTAGCCTTCTCGAAGGGGTGGAGATCTC	CNS-11R: AGATCTCGAGGAGCACAAGAGTGAGTCCTGCA	intron1
CNS-12F: GCGTGCTAGCCCTCGGCTCCCTCTCTGTGTGTT	CNS-12R: AGATCTCGAGGCCTCCTCTGAACAGAACACAA	intron1
CNS-13F: GCGTGCTAGCCGGGTGTGCAGTGGTAGAAGTGT	CNS-13R: AGATCTCGAGGGCCCAACGAATCACTCAACTA	intron1
CNS-14F: GCGTGCTAGCCGAGCGGAGTTAAAGCCAAGGTC	CNS-14R: AGATCTCGAGCCATTATGATGGTCCCTACCTC	intron1
CNS-15F: GCGTGCTAGCCAGTAGGAGGCGCTGCAGAAGGCAA	CNS-15R: AGATCTCGAGGGCTTCCATTCCCACCAAGTCA	intron1
CNS-16F: GCGTGCTAGCCGAGAGGTTCTGTGCCTCTGTTTCTGACC	CNS-16R: AGATCTCGAGACAAAGCCTGGTCCTGGGACTGTCC	intron1
CNS-17F: GCGTGCTAGCCCTTCTTCTGTTTAAATCTCCTAGG	CNS-17R: AGATCTCGAGATGATGACAGGGGTTTCTGAGT	intron1
CNS-18F: GCGTGCTAGCCCTCTCTTGGTGCACACAGTAGGT	CNS-18R: AGATCTCGAGGAAACACCGTCATGGCACACAG	intron1
CNS-19F: GCGTGCTAGCCTGTGTGTTTACACAGAGATGGTCT	CNS-19R: AGATCTCGAGATATGCTGAGTGGCACTTCGCT	intron1
CNS-20F: GCGTGCTAGCCCTTCAGCAGAGCAGCAGAGCTAA	CNS-20R: AGATCTCGAGGCGATCTTGCTAAGTCCTGAAG	intron1

CNS-21F: GCGTGCTAGCCGTGAAATTAACTTGAACACATT	CNS-21R: AGATCTCGAGGCTATTGTAAAGTCACATCCAT	intron1
CNS-22F: GCGTGCTAGCCCAGTGAATGAGTGGCCACCCTG	CNS-22R: AGATCTCGAGTATGCAGCCCAGATGCTCCCAG	intron1
CNS-23F: GCGTGCTAGCCTTTACGAATGGGAAAACTGAGGTAG	CNS-23R: AGATCTCGAGAGAAATGCCCTGAGCCAGAGAA	intron1
CNS-24F: GCGTGCTAGCCGACTGCAAACCCTAGGTGCTGG	CNS-24R: AGATCTCGAGAATCAGGTTACCCTGGGGAGCCTC	intron1
CNS-25F: GCGTGCTAGCCTGAACCTGAGCCCAGAGCATCCTC	CNS-25R: AGATCTCGAGCAAGGGAGCTGCTGAAACCAGA	intron1
CNS-26F: GCGTGCTAGCCTGTACCAGTGAGCCTCTGCAGAT	CNS-26R: AGATCTCGAGTACAGGGCAACCCTCTCCACTG	intron1
CNS-27F: GCGTGCTAGCCATGCATTGATGAGTGTTACAGA	CNS-27R: AGATCTCGAGTAAGAGGCACTCTGTGTCTTTT	intron1
CNS-28F: GCGTGCTAGCCAGCTCGTTGGGGTCATCGGTGA	CNS-28R: AGATCTCGAGCCAAGTGACAGCAGAACCGGAA	intron1
CNS-86F GCGTGCTAGCCAGGGGGAGAAGGGGCGCAGATC	CNS-86R AGATCTCGAGCATATCCAGCCCTGCTCGGTCA	intron1
CNS-76F GCGTGCTAGCCAGGCTGGAAGGTGGGCTTAGTC	CNS-76R AGATCTCGAGATCCTCTCCCCATAAGCTTGAC	intron2
CNS-77F GCGTGCTAGCCTCCCTTGTCAATGGAGCAGAGG	CNS-77R AGATCTCGAGGCATCTCAGGCTGCATAATTCC	intron2
CNS-87F GCGTGCTAGCCGGGGACCAGCAGGTGAGCGAGT	CNS-87R AGATCTCGAGCACGCAGGGACTGGAGGCTTCG	intron2
CNS-29F GCGTGCTAGCCAACCATTTCCCAATAAAGAGGC	CNS-29R AGATCTCGAGTTACGCTGCATCACTCCAATGG	intron3
CNS-71F GCGTGCTAGCCTCCACACGAGACCCTACCAGCA	CNS-71R AGATCTCGAGACCCAGGCTGGTCTCTTTCCCT	intron3
CNS-72F GCGTGCTAGCCACTCAGGCACTCCTTGCTGGCA	CNS-72R AGATCTCGAGGCCTAAAGCATACCCAGGTCCC	intron3
CNS-73F GCGTGCTAGCCAGTGATTCCGCTTGGCCTTGTTCTT	CNS-73R AGATCTCGAGGTGCTATTAAATCAACAGGCAC	intron3
CNS-74F GCGTGCTAGCCGTCCATTGAGCAATGGCTCTGAGGCT	CNS-74R AGATCTCGAGAATGTGCTCCCATCCCCAGGCA	intron3
CNS-75F GCGTGCTAGCCGGCCCCTTGGGAATTTTATAGT	CNS-75R AGATCTCGAGTCAGAGCAAGAAAAGCTGGCCT	intron3
CNS-85F GCGTGCTAGCCAGCAGGAGGAGGAAAGGCCAGCTT	CNS-85R AGATCTCGAGATTTGCCCCACGACCCAGCTCA	intron3
CNS-70F GCGTGCTAGCCACCAAGTATGTCGAGTGAGTATCTTCAGG	CNS-70R AGATCTCGAGAAGTTCCTGGGCCTGGACACAA	intron4
CNS-30F GCGTGCTAGAGGGCAGGCGGTGGTTCTGCTTTGTA	CNS-30R AGATCTCGAGCCCAGGTCCACAAATTCAGTTG	intron5
CNS-66F GCGTGCTAGCCGCAGAGTGGTTGCCTTCTAGCA	CNS-66R AGATCTCGAGCATAGGACTTCGGCTTGAGTGC	intron5
CNS-67F GCGTGCTAGCCTTGCACTCACCCAGAGCCACA	CNS-67R AGATCTCGAGAGGACATGCAGTATATGGGCCA	intron5
CNS-68F GCGTGCTAGCCAGGCTCAGCCAGAGCTCTTCCTGTG	CNS-68R AGATCTCGAGCCCACTGCAGGGAAGCCTTCTT	intron5
CNS-69F GCGTGCTAGCCATCGACCTGAAGAGTGGGGAA	CNS-69R AGATCTCGAGACCTCCTCGGCTCTGACATGAA	intron5
CNS-31F GCGTGCTAGCCTGGTGATAGAAGTAGGGCCAGA	CNS-31R AGATCTCGAGTTGCCAACATCTGTCAAGGAGA	intron6
CNS-32F GCGTGCTAGCCAGACCTGTGAGGTCCACCCACT	CNS-32R AGATCTCGAGAGAGGATGACCTCCCCATGGTG	intron6
CNS-64F GCGTGCTAGCCACAGAAGCCACAGGCGCTCCTA	CNS-64R AGATCTCGAGTTGCCCTGTCCCTCTAGAACCT	intron6

CNS-65F GCGTGCTAGCCTTCAGGTGAAAATCAGGTTAAACAC	CNS-65R AGATCTCGAGGAAAAGCAGTCCACTATCTTGG	intron6
CNS-63F GCGTGCTAGCCCTGAGTGGTTTCGGGTAGGGT	CNS-63R AGATCTCGAGATCCTGCTGCCAGTGTCCCCAT	intron7
CNS-62F GCGTGCTAGCCTCCCTAGGCTATGGCTATTTG	CNS-62R AGATCTCGAGAGATCTTCCCTGCGGACCTGAG	intron9
CNS-84F GCGTGCTAGCCGGAGCGCCTCTATCAGCAGGTGTGT	CNS-84R AGATCTCGAGTTGTCCCCTGTCCCCTCCAAAG	intron9
CNS-61F GCGTGCTAGCCTGCCCACTTTAGCCACTCCTAT	CNS-61R AGATCTCGAGCACACCTACCCCAAAATGAGGA	intron12
CNS-33F GCGTGCTAGCCGGTCAGCAGCTGGGAGCGCTAATA	CNS-33R AGATCTCGAGGCCCAACCCTGTGCTTTGTTTT	intron14
CNS-58F GCGTGCTAGCCTCACAGGGAAATGGCTAAACCA	CNS-58R AGATCTCGAGCTTCCATGCCAATTAAACACAA	intron14
CNS-59F GCGTGCTAGCCAGTTGTGTTATAGCTATATTTTGTTCTG	CNS-59R AGATCTCGAGGCTTTTCCCATGCTGATTATTT	intron14
CNS-60F GCGTGCTAGCCAGCTGGGTGTAGTAAGTAAATC	CNS-60R AGATCTCGAGCAGCTATCATTTGTTTTGAGTG	intron14
CNS-83F GCGTGCTAGCCAAAAGGTCATGGGAATGGAATA	CNS-83R AGATCTCGAGTGGAGATGAAATTGTGTAGTCA	intron14
CNS-91F GCGTGCTAGCCGCACTAGTTTCCTCTCCTCATTC	CNS-91R AGATCTCGAGTGGTCACAACACTTTGCACATG	intron14
CNS-82F GCGTGCTAGCCAGACTGTCTATTCTGGGGAGAATGAG	CNS-82R AGATCTCGAGATGCCCACCCACAGAGTCTAGG	intron16
CNS-57F GCGTGCTAGCCCTCCAGGACTGGCAAGGTAGA	CNS-57R AGATCTCGAGAGAAGAAGCCATCTGAGCCTGG	intron17
CNS-34F GCGTGCTAGCCCAAAATGTGTGGGTGGGGTTGC	CNS-34R AGATCTCGAGCAGGCATGTCACATCCAGAACA	intron18
CNS-56F GCGTGCTAGCCTGGAAATTCTGGTCCCAAACTCT	CNS-56R AGATCTCGAGAAGTCTTCTTTGGATGGGGAGA	intron18
CNS-35F GCGTGCTAGCCTGCCTCGCCTCTGTTCCAGAAT	CNS-35R AGATCTCGAGCCTTTTGCTGTGAGATCTCTGT	intron20
CNS-36F GCGTGCTAGCCTTGGGTCACTGTCGCTCACAGAG	CNS-36R AGATCTCGAGCCAGACTGTGCTATGAGGCTCC	intron20
CNS-51F GCGTGCTAGCCAGAACTACATAAACAGATCTGA	CNS-51R AGATCTCGAGGGTTTCATTGTTTTACATTACA	intron20
CNS-52F GCGTGCTAGCCAACTGACTCTCAAATTAACTTA	CNS-52R AGATCTCGAGTTAATTAATTACACTGGCTAGT	intron20
CNS-53F GCGTGCTAGCCCCATATGATCTGCAGTAATAA	CNS-53R AGATCTCGAGTTGGGGATGTGAGAAATTAGAT	intron20
CNS-54F GCGTGCTAGCCTCACAGCAAAAGGTAGAAGGA	CNS-54R AGATCTCGAGATTTGAAAGCACATGGCTGAAG	intron20
CNS-55F GCGTGCTAGCCACGTATGGAGGCCAGAGCAGGA	CNS-55R AGATCTCGAGTGTAGGAGGCTGGTGATCTGGG	intron20
CNS-37F GCGTGCTAGCCGAGACACACATAGCTTATTCCTGGTGGG	CNS-37R AGATCTCGAGAGTTTGCCCTTGGCTGGCTCTG	intron22
CNS-50F GCGTGCTAGCCATCTGGGCTGATTTACAATTAAAGACAC	CNS-50R AGATCTCGAGTGCTTCCCTTTTAGTTCCCACA	intron26
CNS-38F GCGTGCTAGCCGTTAAAACCACTCAACAGCCCC	CNS-38R AGATCTCGAGCCTGGTTGAACTTGGGATATAT	3'-end
CNS-39F GCGTGCTAGCCCTGGTTGCTAGGGCCTGTCTA	CNS-39R AGATCTCGAGTGTCCAGCATCCCTCCTCTTTG	3'-end
CNS-40F GCGTGCTAGCCGATAGAGGAGATGGGGTGAGGA	CNS40R AGATCTCGAGACAGACCTCCCTCAGTCCTTTC	3'-end
CNS-41F GCGTGCTAGCCAGCAACATCATTCTTCAAATAACTGGC	CNS-41R AGATCTCGAGTTCCAGGGCCACTCATTGTATT	3'-end

CNS-42F GCGTGCTAGCCCCTCACCATTGTATCCAAATG	CNS-42R AGATCTCGAGAACTTACTAGTTGGCCCATTCA	3'-end
CNS-43F GCGTGCTAGCCTTATCTGGAGAACAGACCTCC	CNS-43R AGATCTCGAGTGACCTCATTTCCCTTTTCTTG	3'-end
CNS-44F GCGTGCTAGCCAGCCTCCCAGGCCTTCACTTCCACT	CNS-44R AGATCTCGAGAGGTGGTGGCTGGCCTGGAAGG	3'-end
CNS-45F GCGTGCTAGCCTGCCTTCATCCCTCCCTGACTCT	CNS-45R AGATCTCGAGTGTCCACTCCCCGAGTCACATC	3'-end
CNS-46F GCGTGCTAGCCCTCCCTGTTTAAGAGAAGCCGG	CNS-46R AGATCTCGAGTGAGACAGAGACCGAAGGGAGG	3'-end
CNS-47F GCGTGCTAGCCGGAAGCGCCATCCTCATGTGGGTA	CNS-47R AGATCTCGAGTGCACACTCCCTGATGCTCTGC	3'-end
CNS-48F GCGTGCTAGCCAGCAAGGCTGCAGCTGTCACTG	CNS-48R AGATCTCGAGCACCACACCCTTGCCTCACTCT	3'-end
CNS-49F GCGTGCTAGCCCTTGGGTGAGGCCAGCATATC	CNS-49R AGATCTCGAGAGTGTTCTGGCAAGGCTGGGAG	3'-end
CNS-80F GCGTGCTAGCCTACCTAGGACTTATCCCACAGA	CNS-80R AGATCTCGAGTACCATTCTGTTTTGTCACACT	3'-end
CNS-81F GCGTGCTAGCCTGACCCCTCAACTTTCCTTTT	CNS-81R AGATCTCGAGCCTGTCTTTTCCGAGTGGTATTA	3'-end
CNS-92F GCGTGCTAGCCACAGGGAGATGCCTCAAATCC	CNS-92R AGATCTCGAGGGCAACTGCCAGCCTTTAAGTA	3'-end

Online Table II. Echocardiogram Measurements of Adult Mice

	H/H (n=9)	CNS28$^{-/-}$ (n=5)
Septal Wall (mm)	0.72 ± 0.02	0.74 ± 0.05
Posterior Wall (mm)	2.98 ± 0.11	3.06 ± 0.19
Left Ventricle Systole (mm)	0.58 ± 0.02	0.52 ± 0.03
Left Ventricle Diastole (mm)	1.50 ± 0.06	1.65 ± 0.11
Fractional Shortening %	49.7 ± 0.9	46.0 ± 1.9

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