Supporting Information

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SI Text

Materials and Methods. ***Plasmid construction.*** The pCMV-*piggyBac* and pT-NeomycinR (formerly referred to as pTpB) plasmids were described previously (13). All other plasmids were constructed as described using standard recombinant DNA techniques using enzymes from New England Biolabs (Ipswich, MA). Each plasmid construct was verified by DNA sequencing. Fig. S1 illustrates the composition of all transposons used in this study. All plasmids are available from the authors upon request. The pCAGGS-Luciferase plasmid was generated using the luciferase cDNA from pGL3-basic (Promega, Madison, WI) and the β-actin promoter/cytomegalovirus enhancer (CAGGS) from pCAGGS-Cre (kindly provided by Earl Ruley, Vanderbilt University). pT-Luciferase was constructed by subcloning the inverted repeat elements from pT-NeomycinR into pCAGGS-Luciferase. To construct pT-SCN1B:cMyc–IRES–SCN2B:HA, SCN1B and SCN2B cDNAs(23) were sequentially subcloned into the pIRES vector (Clontech, Mountain View, CA), then c-Myc and HA tags were added in-frame to the SCN1B and SCN2B C-termini, respectively, by recombinant PCR mutagenesis. The neomycin resistance gene was exchanged with a puromycin resistance gene derived from pIRESpuro3 (Clontech, Mountain View CA), and *piggyBac* transposon inverted repeat elements were then subcloned from pT-NeomycinR. To create pT-SCN1A:3XFLAG, the *piggyBac* inverted repeats were sequentially subcloned into the previously described plasmid encoding SCN1A-3XFLAG (23). To create pT-SCN1A:Venus, the 3XFLAG epitope in pT-SCN1A:3XFLAG was replaced by Venus (generously provided by David Piston, Vanderbilt University). Reporter gene plasmids pT-eGFP and pT-DsRED were generated by subcloning XhoI/NotI fragments from pIRES2-eGFP or pIRES2-dsRED (Clonetech, Mountain View, CA) into pT-SCN1B:cMyc–IRES–SCN2B:HA. To create pT-CD8, the inverted repeats from pT-NeomycinR were subcloned into the previously reported bicistronic plasmid encoding CD8 and SCN2B(23). The neomycin resistance gene was then removed by PvuII digestion followed by plasmid recircularization.

Stable Expression from Multiple Transposons. For demonstrating stable expression of two independent transposons, 1×10^6 HEK-293 cells were cotransfected with 1 μg each of pCMV-*piggyBac*, pT-NeomycinR, and pT-Luciferase (or pCAGGS-Luciferase as a control) using FuGENE-6 (Roche Diagnostics, Indianapolis, IN) as described previously (13). Cells stably expressing pT-NeomycinR were selected for 3 weeks in growth media supplemented with 800 μg/mL G418. To assay luciferase expression, luciferin substrate (Caliper Life Sciences, Hopkinton, MA) was added directly to the cell culture media according to the manufacturer's instructions, and culture plates were imaged using a Xenogen IVIS imaging system (Caliper Life Sciences, Hopkinton, MA). For demonstrating stable expression of multiple transposon-encoded reporter genes, HEK-293 cells were cotransfected with 1 μg each of pCMV-*piggyBac*, pT-NeomycinR, pT-eGFP, pT-DsRed, and pT-CD8. Cells were selected for 3 weeks in growth media supplemented with 800 μg/mL G418, and coexpression of eGFP, DsRed, and CD8 was quantified by flow cytometry.

Flow cytometry. Transfected and selected HEK-293 cells were passaged with trypsin then placed in phosphate buffered saline (pH 7.4) supplemented with 1% fetal bovine serum (Invitrogen, Carlsbad, CA). A minimum of 10,000 cells were analyzed for reporter gene expression using a FACSAria II instrument (Baylor Cytometry and Cell Sorting Core Facility) and FlowJo analysis software (Ashland, OR). Reporter genes eGFP and DsRed were detected using 488 nm and 561 nm lasers, respectively. The extracellular CD8 antigen was analyzed using anti-CD8 monoclonal antibodies (1:100 dilution) conjugated with allophycocyanin (APC) according to the manufacturer (Beckman Coulter, Fullerton, CA) and detected using a 638 nm laser. Individual cells were determined and subsequently evaluated using standard forward and side scatter analysis. Control cells transfected with single reporter genes alone were used for determining and compensating for potential spectral overlap. The threshold for detection of each reporter gene was determined by direct comparisons with non-transfected control cells analyzed on the same day as the experimental transfected cells.

Stable Expression of Human Brain Sodium Channels. For demonstrating stable expression of a multiprotein complex, 1×10^6 HEK-293 cells were cotransfected with 0.8 μg of pT-SCN1A:3XFLAG (or the combination of 0.4 μg pT-SCN1A:3XFLAG and 0.4 μg pT-SCN1A:Venus), 0.4 μg of pT-SCN1B:cMyc-IRES-SCN2B:HA, and 0.5 μg pCMV-*piggyBac*. Cells were selected for 3 weeks in growth media supplemented with 1 mg/mL G418 and 3 μg/mL puromycin. Individual colonies were selected using 3-mm cloning disks, expanded, and assayed for sodium channel expression using immunoblot analysis or electrophysiological recording (see below). For determining stability of expression over time, clonal lines were serially passaged after achieving 80% confluence, then replated at 1:10 dilution to maintain log phase growth. Quantification of colony formation was done using methylene blue staining as described previously (13). Protein expression was verified using immunoblot analysis.

Immunoblot analysis Cells were washed twice with 4°C PBS on ice and lysed with RIPA buffer (150 mM NaCl, 50 mM Tris-base, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.5) supplemented with the Complete Mini protease inhibitor cocktail (Roche, Nutley, NJ). Solubilized lysate containing 30 μg of protein was diluted in 2X Laemmli buffer with 5% β-mercaptoethanol, subjected to 4–20% gradient SDS-PAGE and transferred to a PVDF membrane. Proteins were detected with primary antibodies directed against the appropriate epitope: FLAG (mouse, anti-FLAG M2, 1:15,000, Sigma-Aldrich), Venus (rabbit, anti-GFP, 1:10,000, Invitrogen), c-Myc (mouse, anti-c-Myc 9E10, 1:10,000, Covance, Emeryville, CA) and HA (mouse, anti-HA.11 16B12, 1:10,000, Covance, Emeryville, CA). Immunoreactive bands were detected using horseradish peroxidase-conjugated secondary antibody directed against the primary antibody (goat, anti-mouse, 1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA or goat, anti-rabbit, 1:10,000, Jackson ImmunoResearch, West Grove, PA) coupled with ECL Plus reagent and Hypersensitive ECL film (GE Healthcare Bio-Sciences Corp, Piscataway, NJ).

Electrophysiological recording–Conventional patch-clamp. Conventional whole-cell patch-clamp recording was used to measure functional expression of SCN1A channels as described previously (23). Sodium currents were recorded at room temperature with an Axopatch 200 amplifier (Molecular Devices, Sunnyvale, CA). Patch pipettes were fabricated from borosilicate glass (Warner Instrument Co., Hamden, CT, U.S.A) and had resistance was between 1.0 and 1.5 MΩ. The internal solution consisted of (in mM) 110 CsF, 10 NaF, 20 CsCl, 2 EGTA, 10 HEPES, with pH 7.35 and

osmolarity 300 mOsmol/kg. The external solution consisted of (in mM): 145 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 dextrose, 10 HEPES, with pH 7.35 and osmolarity 310 mOsmol/kg that was adjusted with sucrose. Cells were allowed to stabilize for 10 min after establishment of the whole-cell configuration before current was measured. Series resistance was compensated (90%, 10 μs) to assure that the command potential was reached within microseconds with a voltage error <2 mV. Leak currents were subtracted by using an online P/4 procedure. Whole-cell currents were low-pass Bessel filtered at 5 kHz and digitized at 50 kHz.

Automated planar patch-clamp recording. Whole-cell planar patch-clamp recording was used to demonstrate stable SCN1A sodium channel expression in cell lines for high-throughput analysis using an automated electrophysiology platform (NPC-16 Patchliner system, Nanion Technologies GmbH, Germany). Patchcontrol HT software (HEKA Electronik, Germany) was used to control application of solutions and pressures necessary to establish the whole-cell configuration. Patchmaster software (HEKA) was used to automatically compensate for whole-cell capacitance and series resistance, and perform voltage-clamp protocols. Cells were detached with 0.25% trypsin-EDTA (invitrogen, Carlsbad, CA) for 1 min at room temperature, then triturated in growth media, centrifuged ($100 \times g$, 2 min) and resuspended at 1×10^6cells/mL in fresh growth media. Following a recovery period (30 min, 37°C) the cells were resuspended and placed in the Patchliner 'cell hotel'. Sodium currents were recorded at room temperature using external and internal solutions identical to those used for conventional patch-clamp experiments. Following cell contact with the 2 MΩ planar electrode, seal enhancer solution (in mM: 80 NaCl, 3 KCl, 35 $CaCl_2$, 10 HEPES/NaOH pH 7.4) was added to the external solution to promote giga-ohm seal formation. After establishing the whole-cell configuration, the seal enhancer solution was replaced with fresh external solution and the series resistance was compensated (50%, 10 μs). Cells were allowed to stabilize for 10 min after establishment of the whole-cell configuration before current was measured. Leak currents were subtracted by using an online P/4 procedure. All whole-cell currents were low-pass Bessel filtered at 5 kHz and digitized at 50 kHz.

Electrophysiological data analysis. Whole-cell conductance was calculated from the peak current amplitude by $G_{Na} = I_{Na}/(V\text{-}E_{Na})$ and normalized to the maximum conductance between −80 and +20 mV. Conductance-voltage curves were fit with a Boltzmann function to determine the voltage for half-maximal activation ($V_{1/2}$) and a slope factor (k). Data analysis was performed using Clampfit 9.2 (Axon Instruments, Union City, CA), Excel 2007 (Microsoft, Seattle, WA), and OriginPro 7.0 (OriginLab, Northampton, MA) software. Results are presented as mean-SEM.

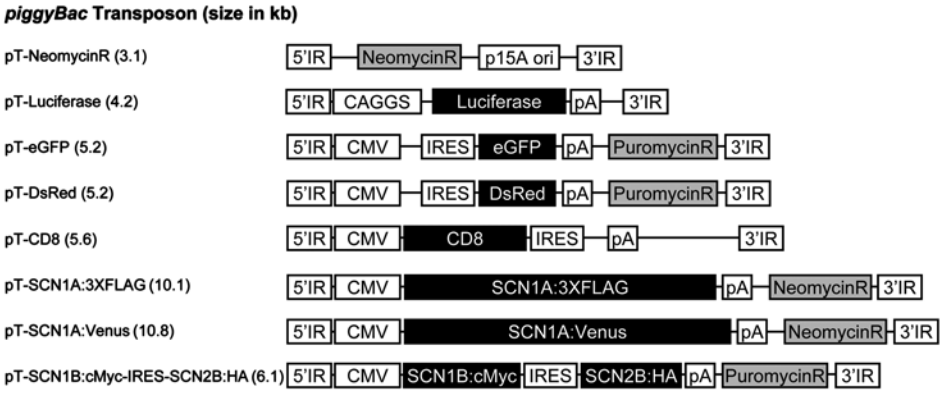

Fig. S1. Constructed *piggyBac* transposons. All *piggyBac* transposons were constructed on separate plasmids. The transposon cassette for genomic integration is delimited by 5′ and 3′ inverted repeats (5′IR and 3′IR, respectively). Successful genomic integration was monitored by stable expression of either antibiotic resistance (*Gray Boxes*) or transgene protein (*Black Boxes*). p15a is a bacterial origin of replication that has been used previously for plasmid-rescue of genomic integration events (13).

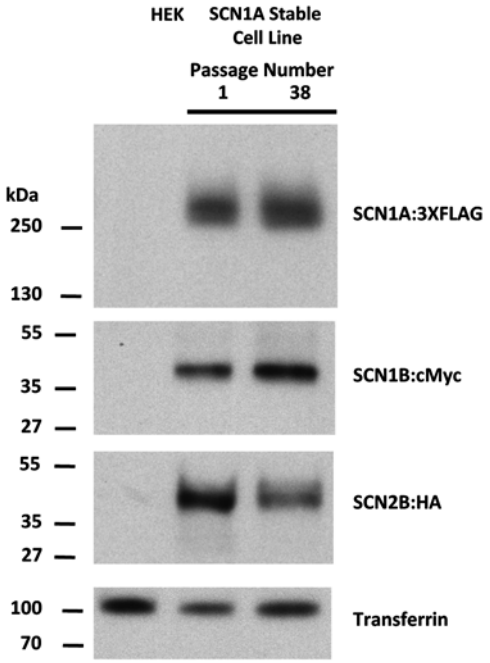

Fig. S2. Protein expression from integrated *piggyBac* transposons exhibits high temporal stability. Protein was isolated from SCN1A clone 1 (HEK cells stably co-expressing pT-SCN1A:3XFLAG and pT-SCN1B:cMyc-IRES-SCN2B:HA) grown in the presence of G418/Puromycin selection. Total protein was isolated at passage numbers 1 and 38 and analyzed by Western blot analysis. Transgene specific proteins were detected by primary antibodies directed against the appropriate epitope tag (FLAG, cMyc or HA). Detection of the endogenous protein transferrin confirms protein loading (10 ug per lane). Total protein isolated from nontransfected HEK cells (left lane, HEK) confirms primary antibody specificity.

Biophysical parameters of SCN1A activation

		Peak Current Density	Activation		
Method	Passage	Step—10mV (pA/pF)	$V_{1/2}$ (mV)	k (mV)	n
Conventional	1	-338–71	-23.7–1.4	-7.3–0.4	5
Conventional	21	-321–58	-23.8–0.9	-6.3–0.3	5
High throughput	4	-402–64	-16.7–1.3	-6.9–0.2	27
High throughput	38	-522–82	-20.3–2.2	-6.1–0.5	21