SUPPLEMENTAL MATERIALS AND METHODS

Creation of the *Scn1a*$^{H939R/+}$ mouse. The *Scn1a<em3Tcp>* mutants were generated by direct delivery of Cas9 reagents to C57BL/6NCrl (Charles River Laboratory, Strain 027) mouse zygotes essentially as in Gertsenstein and Nutter (2018) at The Centre for Phenogenomics (Toronto, ON, Canada). Briefly, a single guide RNA with the desired spacer sequence (Supplemental Table 1) was synthesized by *in vitro* transcription from a PCR-derived template. A microinjection mix of 30 ng/μL Cas9 protein (PNA Bio CP01-50) precomplexed with 20 ng/μL gRNA and 10 ng/μL single-strand oligonucleotide template (Supplemental Table 1, mutated nucleotides indicated in lowercase) was microinjected into C57BL/6NCrl zygotes. Injected zygotes were incubated in KSOM^{AA} media (Zenith Biotech, ZEKS-50) at 37ºC with 6% CO_2 until same-day transfer into CD-1 (Charles River Labs, Strain 022) surrogate host mothers. PCR primers flanking the sgRNA target site (Scn1a primers, Supplemental Table 1) and outside of the repair template homology arms, were used to amplify the region of interest from founder progeny. PCR amplicons were subjected to Sanger sequencing. Founders with the desired nucleotide changes were selected for breeding with C57BL/6NCrl mice to produce N1 progeny that were confirmed by sequence analysis of PCR amplicons using the same PCR primers founder screening.

Mouse Genotyping. At weaning and ear tagging, a small sliver of ear was removed from each pup. Ear clips were digested, and DNA was extracted using the KAPA express buffer and enzyme kit (Sigma Aldrich) in a total volume of 100 μL. Care was taken to ensure no cross-contamination was experienced between samples. The enzymatic solution containing the ear samples was incubated at 75℃ for 10 min followed by enzyme inactivation at 95℃ for 5 min. Extracted DNA was used for genotyping using the QuantaBio PerfeCTa qPCR ToughMix, Low ROX kit according to the manufacturer's instructions. Primers are described in Supplemental Table 2. The PCR mix contained the DNA, forward and reverse primers, a WT probe, em3 probe and the 2x ToughMix in a total volume of 10 μL. The genotyping PCR was run on a BioRad CFX96 Touch Real-Time PCR Detection System with 40 cycles.

SUPPLEMENTAL TABLES

Supplemental Table 1. Sequences used for production of *Scn1a* p.H939R allele.

Name	**Sequence (5'-3')**	**Target site**
Scn1a_gRNA_H939R	AGTCGTTCATGTGCCAACGT	Chr2: 66319523-66319542 (+1)
Scn1a_hdr_H939R	GCATGCAGCTGTTTGGAAAAAGTTACAAAGA TTGTGTCTGCAAAATTGCCACTGACTGCAAA CTgCCACGTTGGCACATGAACGACTTCTTCCg CTCGTTCCTGATCGTGTTCCGCGTGCTGTGTG GGGAGTGGATAGAGACCATGTGGGACTGCAT GGAGGTGGCAGGACAAGCTATGTGCCTTACT GTC	Chr2: 66319419-66319609 (-1)
Scn1a_H939R_F1	TTTCTAGCTCCGAGTGTTCAAGTTG	Chr2: 66319725-66319749 (-1)
Scn1a_H939R_R1	TGACCATCATGAAGACAGTAAGGC	Chr2: 66319406-66319429 (+1)

Supplemental Table 2: Primers and probes for genotyping.

	Forward Primer	Reverse Primer	WT LNA Probe	Em3 LNA probe
Sequence	5'-TGC CAC TGA CTG CAA ACT-3'	5'-TCC CAC ATG GTC TCT ATC CA-3'	5'- /5HEX/A+CG +A+G+T +GGA AG/3IABkFQ/ -3'	5'-/56-FAM/A+CG A+G+C +GGA AGA /3IABkFQ/ -3'
Tm (50mM NaCl)	54.7℃	54.8℃	57.9℃	58.3℃

SUPPLEMENTAL REFERENCES

Gertsenstein M, Nutter LMJ. (2018) Engineering point mutant and epitope-tagged alleles in mice using Cas9 RNA-guided nuclease. Curr Protoc Mouse Biol. 8:28-53.