# The loss-of-function variant p.M764R in the cardiac sodium channel $Na_v1.5$ is associated with ventricular arrhythmias and sudden cardiac death in a family without overt Brugada syndrome

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## Introduction

Sequence data is gaining importance to guide diagnostic work-up as well as tailor treatment. Often genetic variants are designated as variants of uncertain significance (VUS), which is a barrier to the use of sequence data in clinical care.[1]

Loss-of-function variants in the *SCN5A* gene encoding the cardiac sodium channel $Na_V1.5$ have been associated with Brugada syndrome (BrS). BrS is characterized by an electrocardiographic typical repolarization pattern in the right precordial leads ($V_1$–$V_3$).[2] Although penetrance and disease expression vary, the disease is associated with an increased risk of ventricular arrhythmias and sudden cardiac death (SCD).[2,3]

In this study, we describe a family harboring a genetic variant c.2291T>G in the *SCN5A* gene resulting in p.Met764Arg (M764R) in $Na_V1.5$. The variant was found in relatives experiencing ventricular arrhythmias, SCD, and aborted SCD, but without demonstrating classical BrS repolarization patterns on the electrocardiogram (ECG). In silico prediction programs were inconclusive, but patch clamp studies revealed that M764R results in loss-of-function of $Na_v1.5$ and M764R should be reclassified from VUS to likely pathogenic.

## Case report

In 2001, a 33-year-old male patient was referred to the hospital after 2 syncopal episodes. Besides a diagnosis of alpha-1 antitrypsin deficiency and a prior appendectomy, he was previously healthy and did not take any medication. The patient reported having several family members suffering from SCD. A brother had died suddenly at the age of 2 years and a sister was found dead on the toilet at the age of 37 years after having experienced recurrent syncopal episodes. The autopsy of the sister including sections of the heart was reported to be normal. The patient also had a son who died at 14 days of age during signs of respiratory distress. The patient's father had died of cancer at the age of 67 years but his mother and another sister were alive (Figure 1). Neither had prior episodes of syncope or symptoms of heart disease, although his mother, at an older age, developed left bundle branch block associated with a slightly reduced ejection fraction.

Upon admission, an ECG was normal. Echocardiography showed a borderline enlarged right ventricle but normal systolic function of both ventricles and no signs of valve disease. During an electrophysiological study, ventricular pacing with an S1S1 cycle length of 400 ms induced a monomorphic ventricular tachycardia with a cycle length of 270 ms, left bundle branch block pattern, and inferior axis that terminated spontaneously after 30 seconds before an attempt of entrainment or overdrive pacing could be performed. No reinduction of the arrhythmia was attempted. Coronary angiography was normal. Late potentials detected by signal-averaged ECGs were recorded with 3 out of 3 abnormal parameters (signal-averaged QRS: 176 ms; root mean square voltage of the terminal 40 ms of the signal-averaged QRS: 7.6 μV; and late potentials duration: 62 ms). Cardiac magnetic resonance imaging revealed abnormal signals consistent with fatty tissue in a 15 × 15 mm area in the anterior wall of the right ventricle extending up to the outflow tract. Five endomyocardial biopsies were taken showing regular arranged myocytes with no signs of hypertrophy, inflammation, or fibrosis. Interstitial occurrence of mature adipose tissue was

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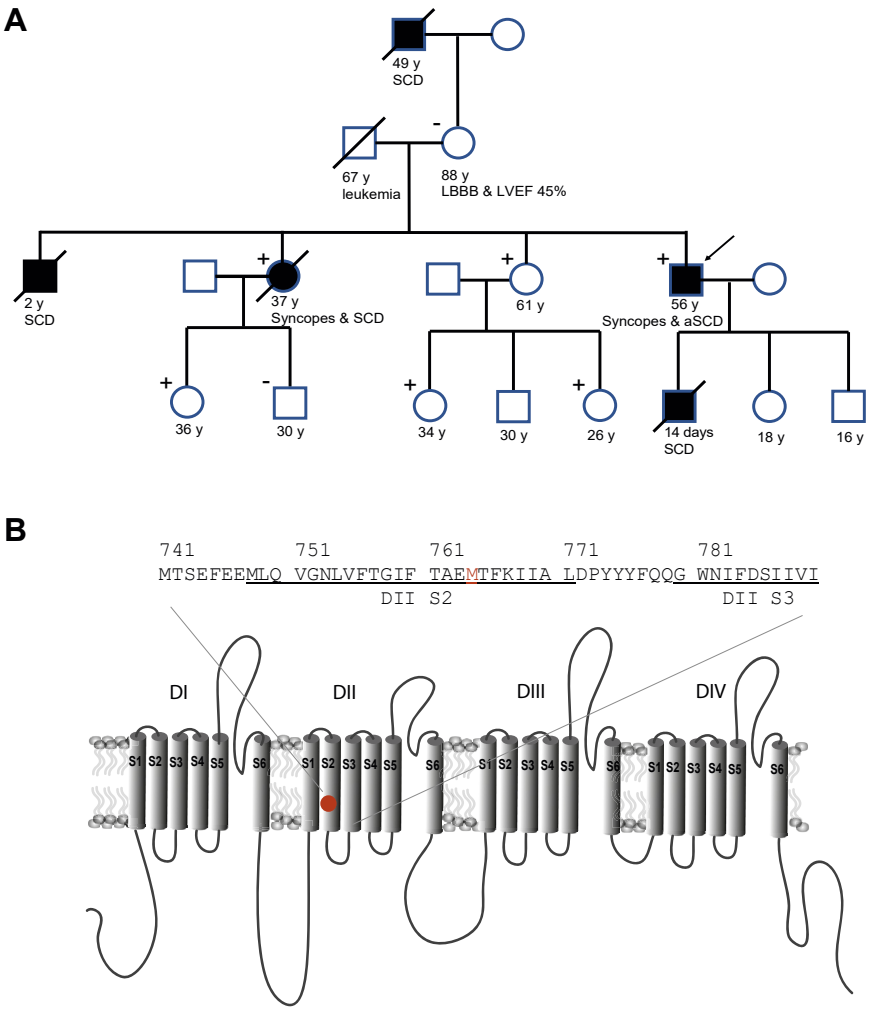


**Figure 1** Family pedigree and position of the M764R variant in $Na_v1.5$. **A:** Family pedigree in 2023. The proband is indicated by arrow. Absence (-) or presence (+) of the *SCN5A* variant (c.2291T>G) resulting in the amino acid substitution M764R is indicated for the family members that underwent genetic testing. aSCD = aborted sudden cardiac death; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction; SCD = sudden cardiac death; y = years. **B:** Structure of the Nav1.5 sodium channel demonstrating the position of M764R in the second transmembrane segment (S2) in the second domain (DII).

seen, in places limited by thin connective tissue septae. Based on these observations an initial diagnosis of arrhythmogenic right ventricular cardiomyopathy was made and a single-chamber implantable cardioverter-defibrillator (ICD) was implanted. Beta-blocker therapy was not commenced. Sanger sequencing of suspected candidate genes, including *PKP2*, *DSG2*, *DSC2*, *DSP*, and *JUP*, did not reveal any variants of interest. Furthermore, the histological sections of the heart from the autopsy on the deceased sister were reevaluated by an experienced cardiac pathologist, without the identification of significant pathologic findings.

At the age of 36 years the patient was admitted because of a syncope during exercise with subsequent appropriate ICD therapy and beta-blocker treatment was initiated. Despite this, the patient was repeatedly admitted again at ages 43 and 45 years because of exercise-induced syncopal episodes with appropriate ICD therapy. During the latter admission, a slow idioventricular rhythm with a rate of 100 beats/min was observed. An unsuccessful attempt of antitachycardia pacing was performed followed by successful DC cardioversion. Echocardiography showed a reduced left ventricular ejection fraction of 45% that normalized within a day of sustained sinus rhythm.

Two years later, in 2013, ongoing atrial flutter was registered on the ICD home monitoring. The patient was admitted and subacute radiofrequency ablation of the cavotricuspid isthmus was performed with successful termination of the flutter. An ECG obtained during sinus rhythm showed a newly developed left anterior fascicular block (Figure 2A) but with stable echocardiographic measurements. Subsequent Holter monitoring showed several short episodes of atrial fibrillation after exercise, but because of limited symptoms no additional therapy was initiated.

In 2015, at 48 years old, the patient experienced cardiac arrest with ventricular fibrillation during sport activities. Unfortunately, ICD therapy failed owing to a short circuit between the transvenous and ICD coils. Immediate cardiopulmonary resuscitation was initiated and return of spontaneous circulation was obtained after 25 minutes, but the episode caused a protracted hospital admission, ending up

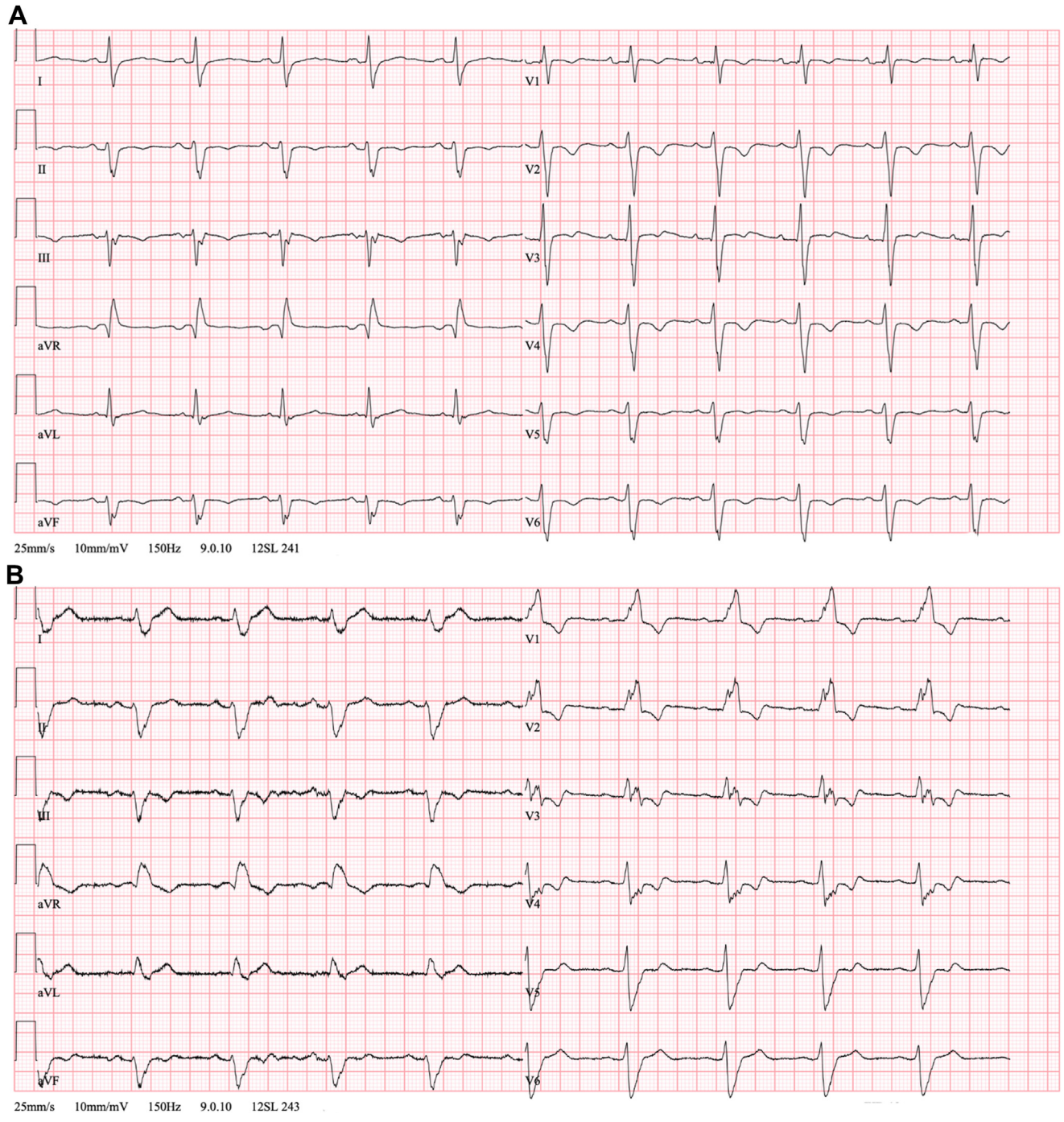


**Figure 2** Electrocardiogram (ECG) of the proband. **A:** In 2015 the proband presents with an ECG demonstrating a left anterior fascicular block. **B:** ECG from the proband in 2022 demonstrating right bundle branch block.

with the extraction and implantation of a new single-chamber ICD followed by discharge to rehabilitation.

In the same year, prior to the cardiac arrest the patient was offered a new genetic evaluation by next-generation sequencing technique using a panel of 12 candidate genes (MOMAs Heart Panel v2, Department of Molecular Medicine [MOMA], Aarhus University Hospital, Denmark). This investigation revealed a heterozygous VUS in *SCN5A* (NM_198056.2): c.2291T>G (M764R), which is a highly conserved nucleotide and amino acid. Methionine is neutral whereas arginine is a positively charged amino acid. In silico predictions using SIFT, PolyPhen-2, and MutationTaster were inconclusive, whereas Varsome indicated the variant is likely pathogenic. The variant is not seen in gnomAD (2.1.1) but is known from ClinVar without classification. In the literature, the variant has been reported in a compound heterozygous form with another VUS (c.1895C>T) in a patient with BrS.[4] A variant at the same nucleotide position as

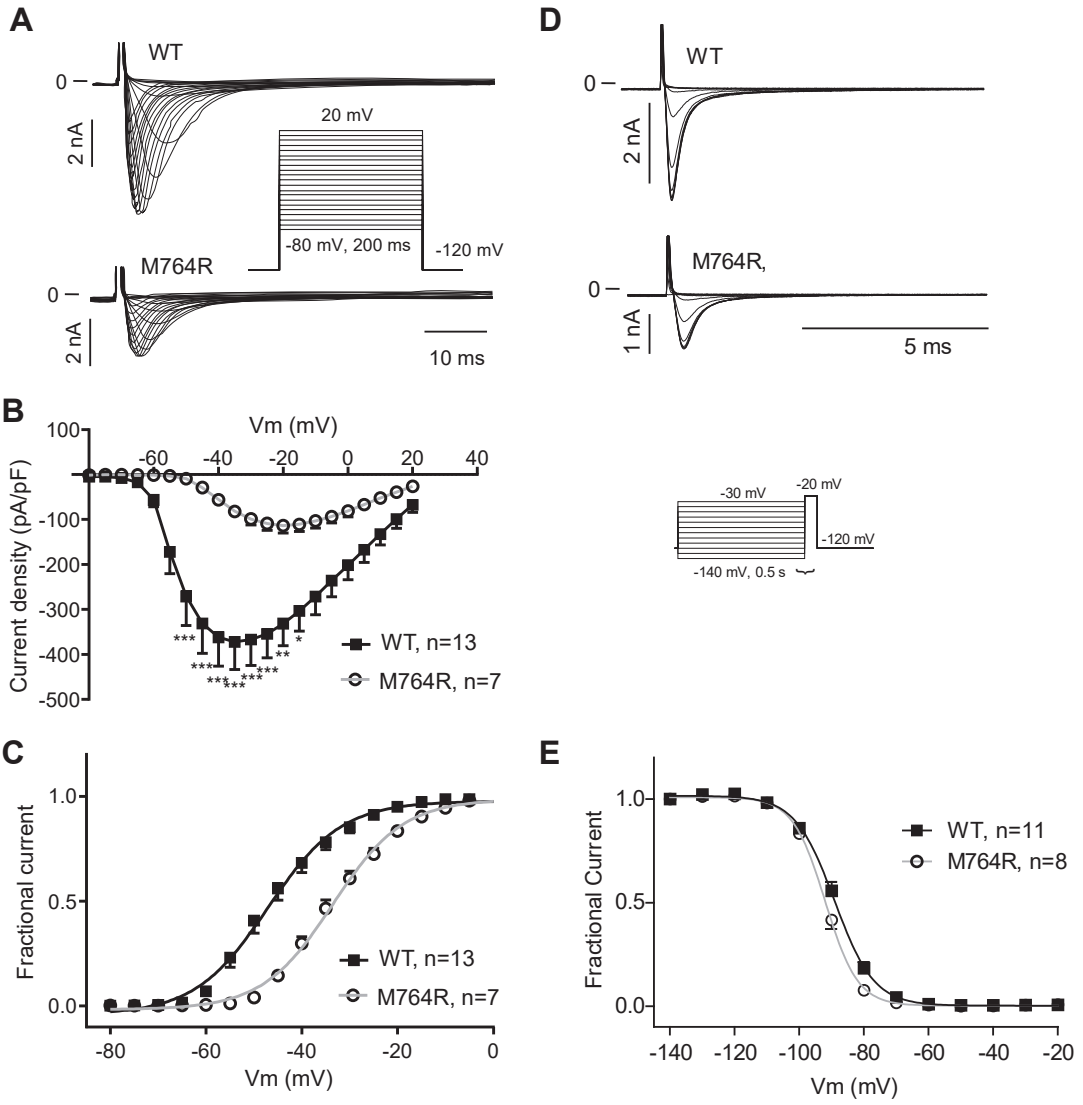


**Figure 3** M764R reduce $Na_v1.5$ current. Whole-cell current recorded from CHO-K cells expressing $Na_v1.5$ wild-type (WT) or M764R. **A:** Representative recordings showing the voltage dependence of activation. **B:** Peak current density as a function of voltage. Significance was tested using a 2-way ANOVA and a Bonferroni post hoc test. **C:** The activation curves were calculated from the data in panel B. **D:** Representative recordings of steady-state inactivation. **E:** Normalized current density recorded at the -20 mV voltage step (indicated by curly bracket) as a function of voltage.

this (c.2291T>A, M764K) has been found in 2 families with BrS.[1,5] This indicates that M764 variants may be connected to BrS. Yet none of the ECGs obtained during the follow-up period showed BrS pattern in the M764R proband and cardiac arrest was associated with exercise. Owing to the development of right bundle branch block (Figure 2B) the patient could not undergo subsequent ajmaline challenge testing. Postmortem genetic testing of the deceased sister and her daughter, as well as genetic testing of the living sister and her daughters, has revealed they all harbor the M764R genetic variant (Figure 1). The living sister as well as her daughter both have normal ECGs, echocardiography examinations, and flecainide/ajmaline challenge tests. To date, none of them has experienced any syncopal events or documented arrhythmias on Holter monitoring.

## Electrophysiological characterization of $Na_v1.5\_M764R$

The M764R substitution is located in the second domain of the $Na_v1.5$ channel in the second transmembrane segment (S2). The transmembrane segments S1–S4 are fundamental for the voltage sensitivity of the channel (Figure 1B). Peak current density was significantly reduced for M764R (Figure 3A and 3B). Compared to wild-type (WT), M764R required more positive voltages for activation; the half-maximal ($V_{50}$) activation was determined from the activation curves and revealed a positive shift for M764R; for WT $V_{50}$ was -46.5 ± 1.7 mV, n = 13 and for M764R $V_{50}$ was -33.5 ± 1.1, n = 7, $P < .0001$ (Figure 3C). There was a small shift in steady-state inactivation (Figure 3D and 3E). For WT $V_{50}$ was -89.1 ± 0.4, n = 11 and for M764R $V_{50}$ was -92.0 ±

0.3, n = 8, ($P < .0001$). The time dependence from recovery was also not significantly affected (data not shown).

## Discussion

In this study we present a family in which several relatives experienced ventricular arrhythmias and/or SCD. Genetic testing and electrophysiological characterization revealed a loss-of-function variant in the *SCN5A* gene at a position previously associated with BrS, but in the absence of Brugada type 1 ECG patterns. Furthermore, 4 genotype-positive female family members did not experience any arrhythmias or repolarization abnormalities on the ECG even during flecainide/ajmaline challenge tests. This finding is also consistent with a low penetrance among females in classical BrS families.[3]

*SCN5A* variants associated with either 50% reduction in peak current or significant gating abnormalities resulting in decreased current (a shift in $V_{50}$ of activation shift of +10 mV, or a shift in $V_{50}$ of inactivation of -10 mV, or the sum is >10 mV) are considered to have a high probability of being pathogenic.[6] $Na_v1.5$_M764R peak currents were reduced with more than 50% and there was a +13 mV shift in $V_{50}$ of activation, indicating that stronger depolarization is needed for $Na_v1.5$_M764R channels to activate compared to WT, which may further suggest that M764R act as a modulator of arrhythmias by promoting unidirectional block owing to lower cellular excitability. The M764R substitution resides in 1 of the voltage-sensing domains of $Na_v1.5$ and the substitution results in replacement of the neutral methionine with a positively charged arginine. The presence of a positive charge in S2 may interfere with movement of S4 and gating of the $Na_v1.5$ channel. Together these results strongly indicate that c.2291T>G p.M764R is likely a pathogenic genetic variant.

## Conclusion

The patients in the present study did not exhibit classical symptoms of BrS, but rather primary ventricular fibrillation and later monomorphic ventricular tachycardia and arrhythmic events were triggered by exercise. BrS could not be unmasked by provocation test using ajmaline/flecainide. All patients carried the M764R substitution, but healthy carriers were found. In silico predictions were in this case not accurate, and patch clamp studies revealed a significant loss-of-function, suggesting M764R should be reclassified from VUS to likely pathogenic.

## Methods

### Genetic analysis

For genetic testing, NimbleGen DNA Capture, Illumina NGS, MLPA, and Sanger sequencing were used. We used a screening panel consisting of 12 genes associated with genetic ion channel cardiac diseases (*CACNA1C, CACNA2D1, CACNB2, GPD1L, KCND3, KCNE1, KCNE2, RANGRF, SCN1B, SCN3B, SCN4B, and SCN5A*).

### Site-directed mutagenesis

Human $Na_v1.5$ isoform 2 (GenBank Acc. No. NM_000335) in pcDNA3 was a kind gift from Dr Charles Antzelevitch. The M764R substitution was introduced and verified (Eurofins, MGW Operon, and GenSript Biotech). Reference sequence is NM198056 the Nav1.5 isoform A, the most abundant in the heart.

### Electrophysiology

Chinese hamster ovary (CHO-K1) cells were transfected with 2 μg $hNa_v1.5$, wild-type (WT) or M764R in pcDNA3 using Lipofetamine 3000 (Thermo Fisher Scientific) according to manufacturer's instructions. Currents were recorded using a MultiClamp 700B amplifier and MultiClamp Commander (Axon Instruments) 2–3 days post transfection and at 22°C–24°C. Extracellular solution (in mM): 130 NaCl, 5 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 2.8 Na-acetate, 10 HEPES, 10 glucose, pH 7.35 with NaOH. Intracellular solution (in mM): 10 KCl, 105 CsF, 10 NaCl, 10 HEPES, 10 EGTA, 5 TEA-Cl, and 4 $Na_2$-ATP, pH = 7.2 with CsOH.

Data were analyzed using pClamp 10.7 software (Axon Instruments, Molecular Devices) and Prism (GraphPad Software). All values are presented as mean ± SEM, statistical significance (*$P < .05$, **$P < .01$, and ***$P < .001$) was evaluated as appropriate by 2-way ANOVA followed by Bonferroni post hoc test or by unpaired student *t* test.

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