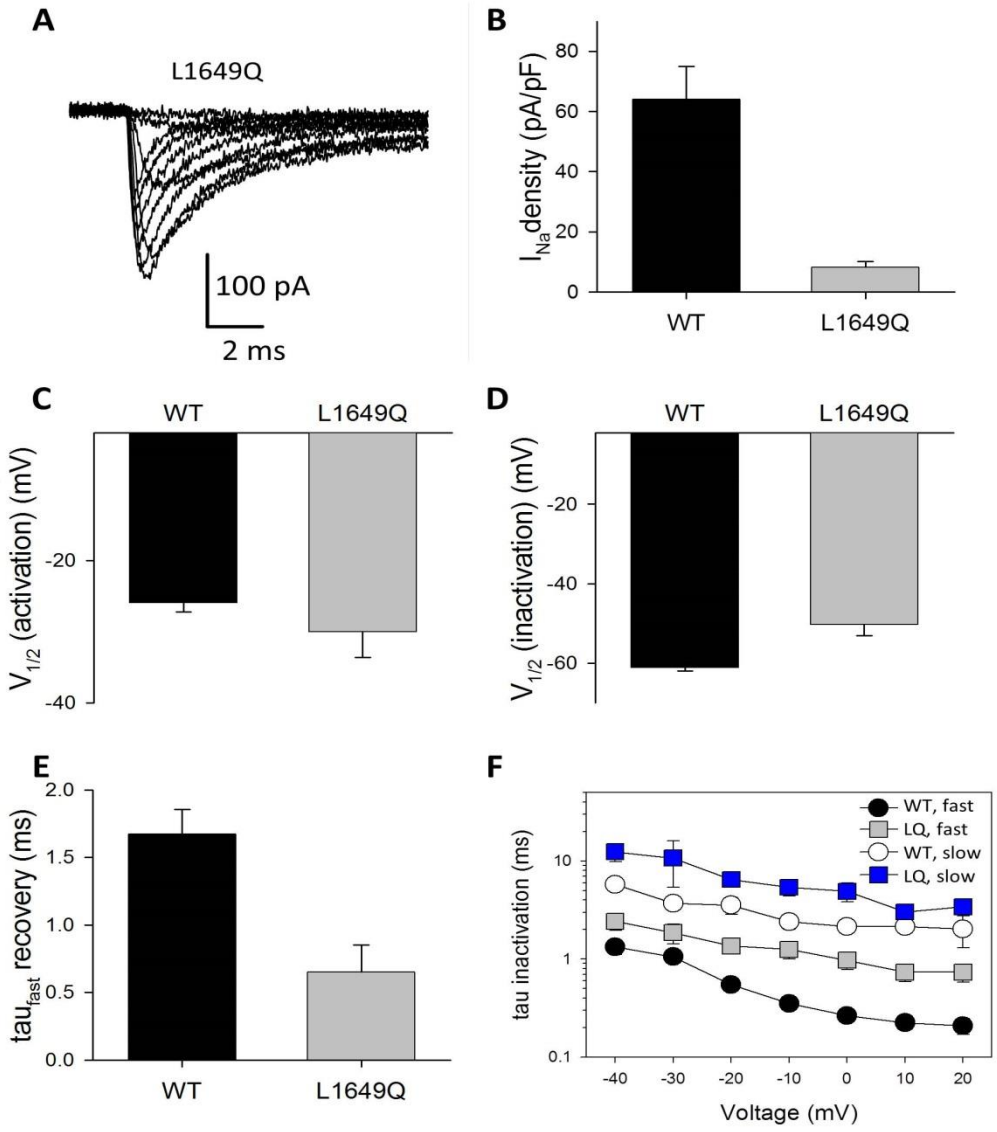
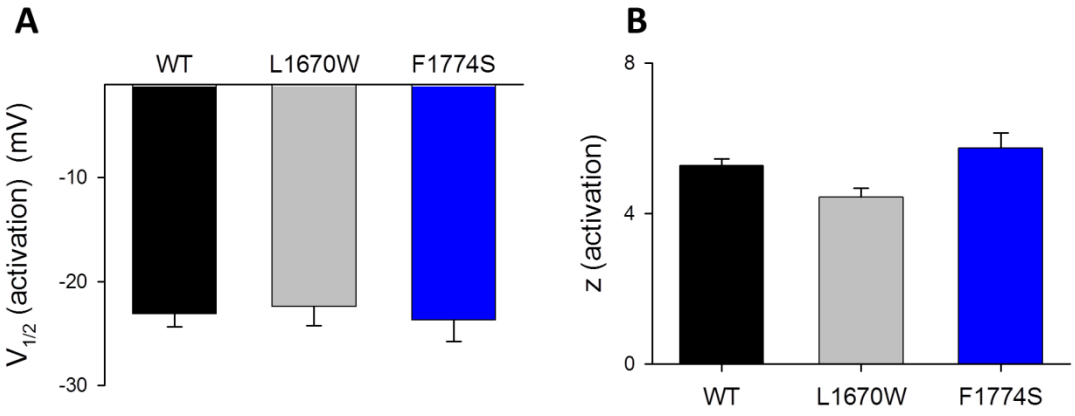
# Supplementary Material



**Supplementary Figure 1 . Functional properties of mutant L1649Q.**

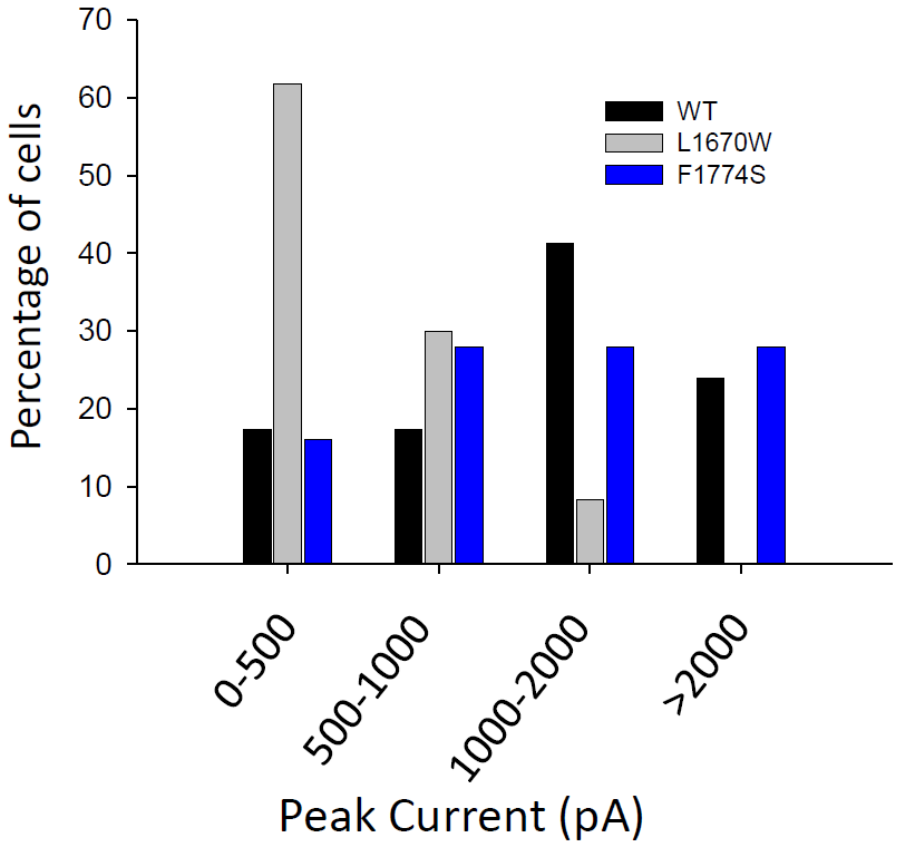
A) Whole-cell $Na^+$ currents recorded from transiently transfected cells with the optimized plasmid carrying the L1649Q mutation. B) Peak current densities recorded from cells expressing the WT optimized plasmid (black, n=11), or the mutant (gray, n=20). Data for WT are the same as in Fig. 3. C, D) Voltages of half-maximal activation (C, difference is not significant, $p>0.2$, Student's unpaired t-test), and half-maximal inactivation (D, values are significantly different, $p<0.05$) obtained for the WT (n=7) or mutant L1649Q (n=6), respectively. Data for WT are the same as in Fig. 3. E) Fast (and dominant) time constant of recovery from inactivation at -120 mV for WT (n=5) and mutant L1649Q (n=4), respectively (values are significantly different, $p<0.05$). F) Fast and slow time constants of inactivation as indicated. Differences between WT and mutant are significantly different at all voltages ($p<0.05$). Note that the reduction in surface expression of the mutant compared to WT is smaller than previously reported [12, 23]. Also the shift of the steady-state inactivation is ~10 mV less than reported [12].



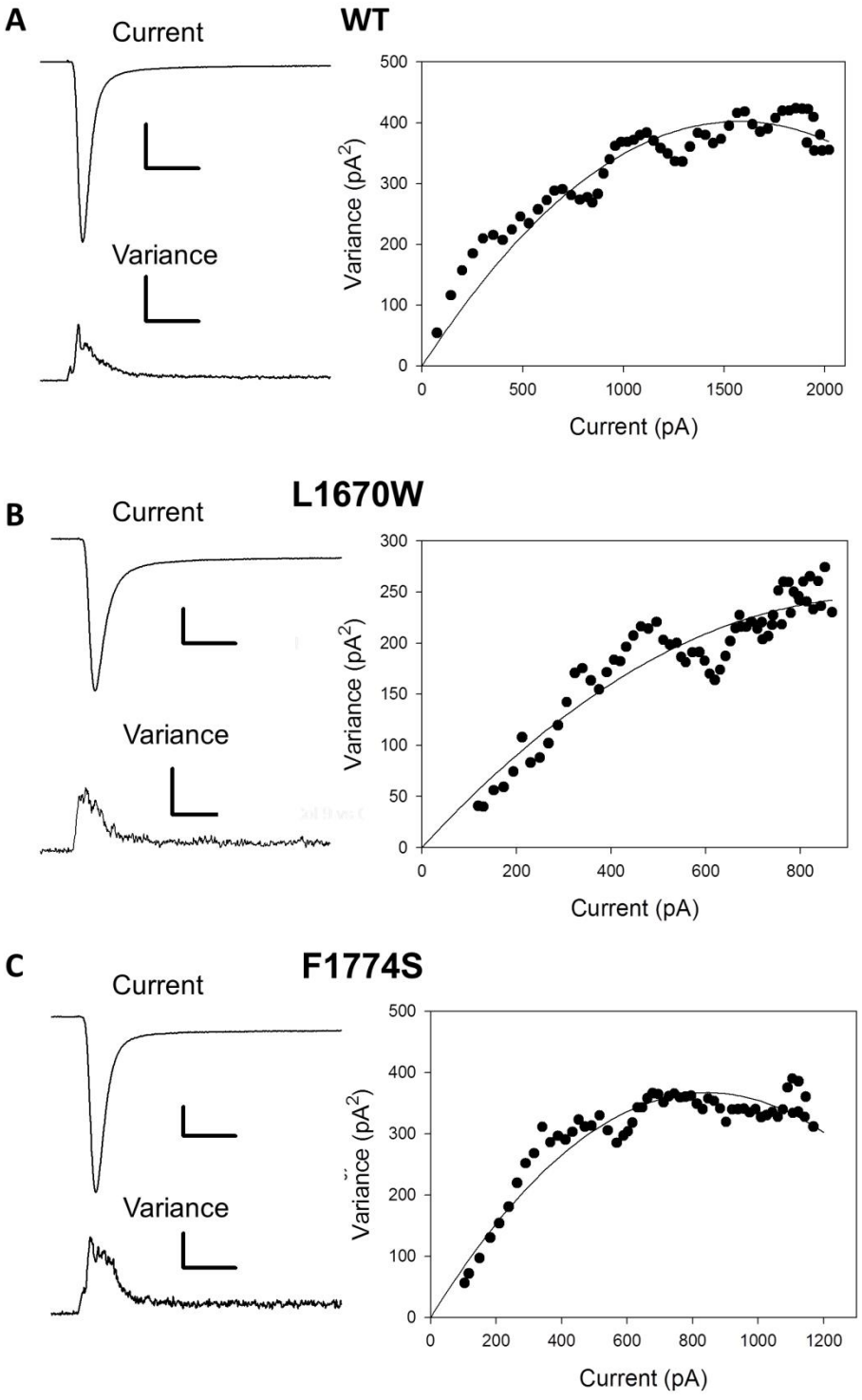

**Supplementary Figure 2. Activation parameters are unchanged for migraine mutations.**

A) Voltage of half-maximal activation (WT: n=17, L1670W: n=21, F1774S: n= 15).

B) Slope factor of the Boltzmann activation curve.

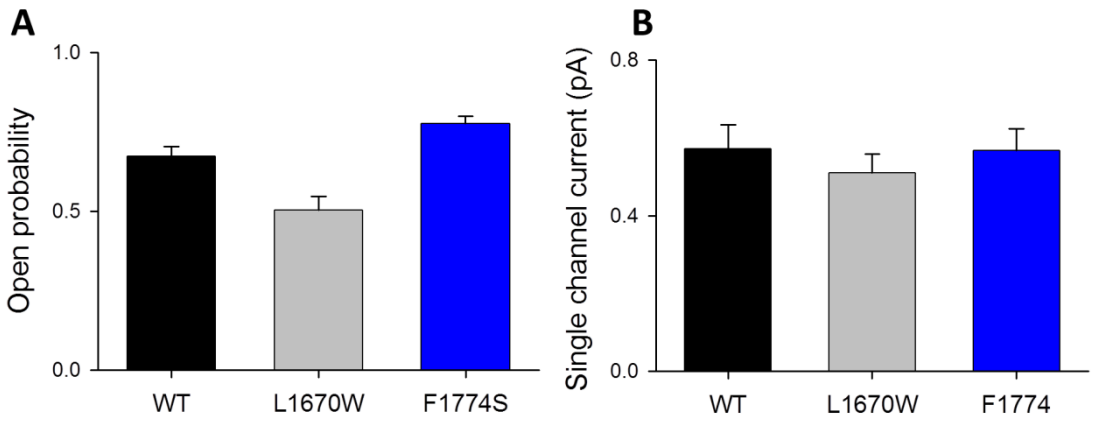



**Supplementary Figure 3. Histogram of expression levels of HEK cells transfected with WT (n=46), L1670W (n=61) and F1774S (n=26) constructs**




**Supplementary Figure 4. Examples of non-stationary noise analysis.**

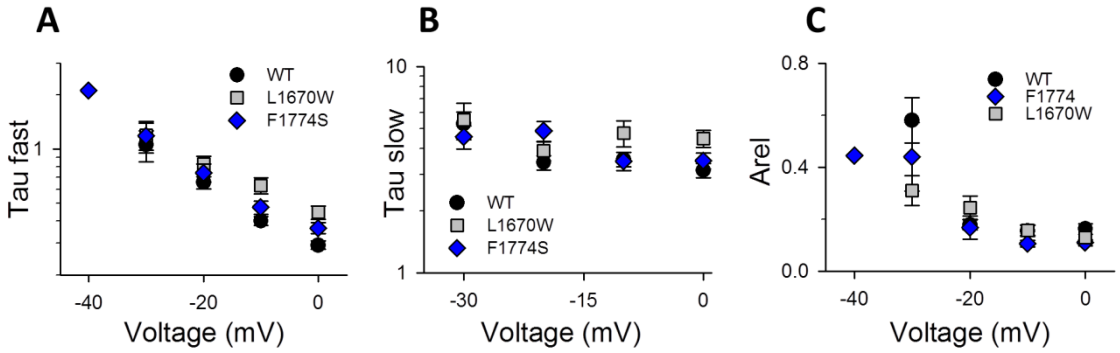
For each construct (WT, L1670W, F1774S), the average response to 200 pulses to 0 mV (top) and the resulting variance trace (bottom) are shown on the left. Variance mean plots (symbols) together with a parabolic fit as described in Methods are shown on the right. Scale bars: horizontal: 2 ms; current scales: A: 500 pA, B: 200 pA, C: 200 pA; variance scales: A: 500 $pA^2$, B: 200 $pA^2$, C: 200 $pA^2$




**Supplementary Figure 5. Results from non-stationary noise analysis.**

A) Peak open probability.

B) Single channel current amplitude. (WT: n=10, L1670W: n=9, F1774S: n= 10).

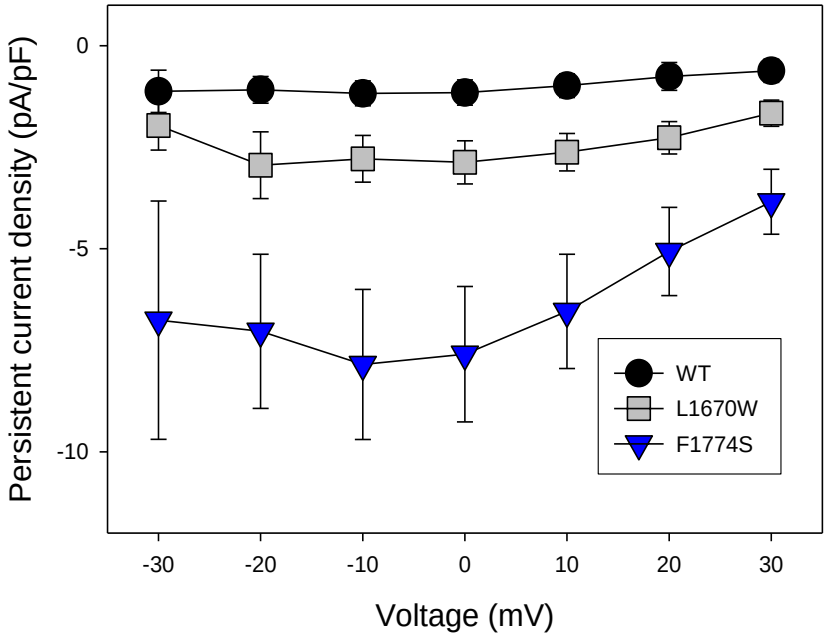



**Supplementary Figure 6. Parameters of fast inactivation kinetics.**

A) Fast time constant of inactivation.

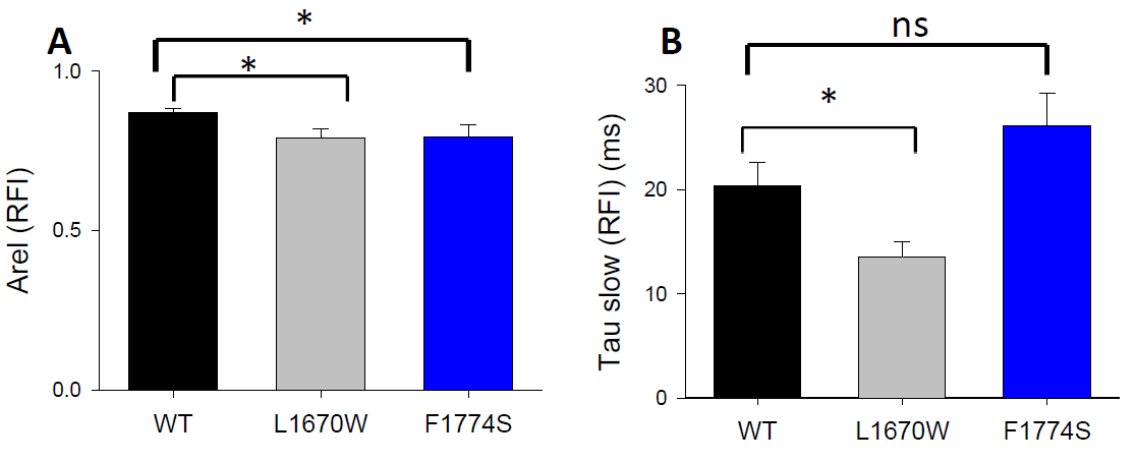
B) Slow time constant of inactivation.

C) Relative area of the slow component of inactivation calculated as described in Methods. (WT: n=13, L1670W: n=17, F1774S: n=12 ).




**Supplementary Figure 7. Absolute persistent current density.**

The persistent current density was calculated by multiplying the relative persistent current (Fig. 5B) with the values of the normalized current voltage relationship (Fig. 4B) and the absolute current density (Fig. 4C). Error bars were calculated by error progression. The differences between WT and both mutations are statistically significant at all potentials ($p < 0.05$, Student's unpaired t-test) except at -30 mV (WT: black circles, n=12; L1670W: gray squares, n=14; F1774S: blue triangles, n=14). The reasoning behind the "back-calculation" is the following: In order to obtain a reliable estimate of the persistent current, stable and relatively large currents, with little leak are required (i.e. measurements shown in Fig. 5B). However, the selection of only these recordings for the determination of the overall density of the persistent current would lead to a bias towards cells with large expression. The bias would be much stronger for the less expressing mutant L1670W. The back-calculation avoids this bias.




**Supplementary Figure 8. Parameters of recovery from inactivation.**

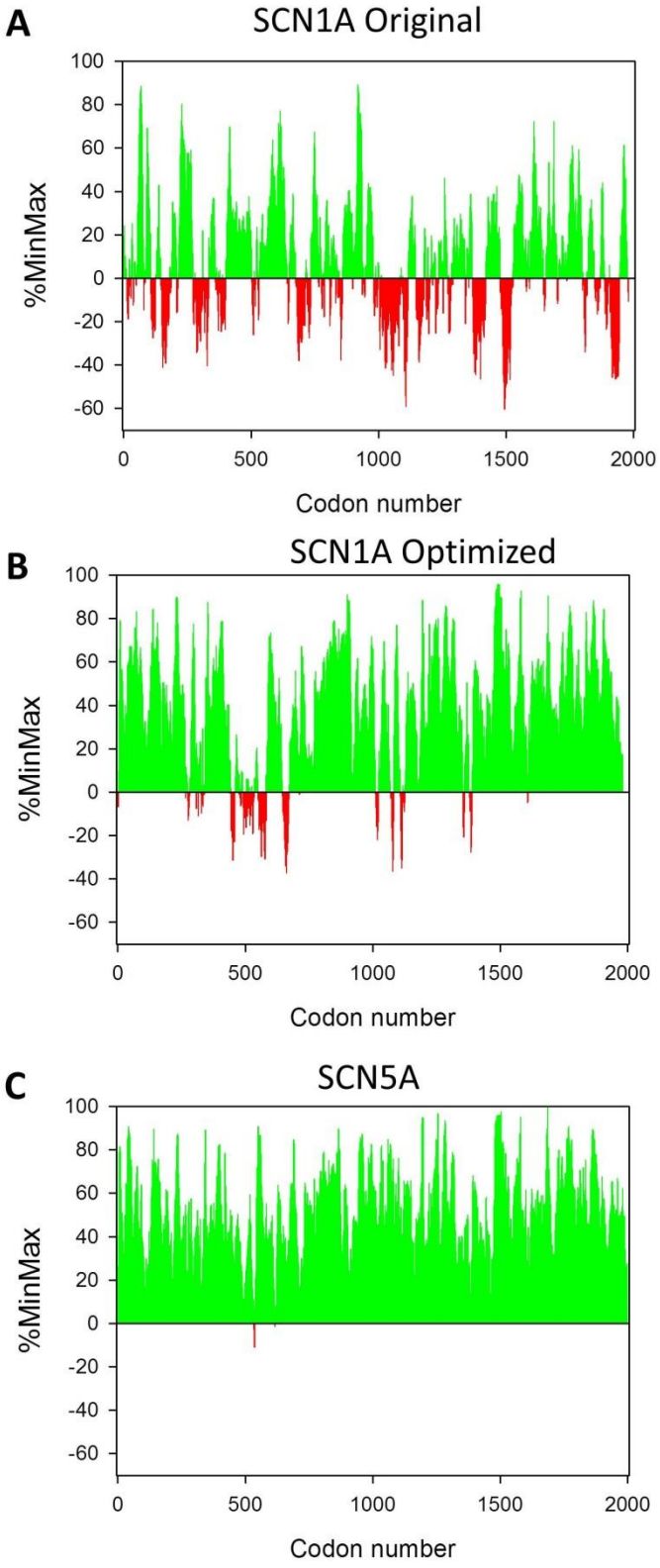
A) Relative area of the slow component of the recovery from inactivation at -120 mV calculated as described in Methods.

B) Slow time constant of recovery from inactivation (WT: n=13; L1670: n=13; F1774S: n=11; asterisks indicate $p<0.05$ comparing WT and mutant, Student's t-test; error bars indicate SEM).

**TABLE 1**

| cDNA | **C**odon **A**daptation **I**ndex (CAI) |
|---|---|
| SCN1A original | 0.756 |
| SCN1A optimized | 0.816 |
| SCN5A | 0.846 |

**The Codon Adaptation Index was calculated using the http://www.codons.org/ online server.**


A
SCN1A Original
%MinMax
Codon number
B
SCN1A Optimized
%MinMax
Codon number
C
SCN5A
%MinMax
Codon number

**Supplementary Figure 9. Codon usage of optimized sequence.**

Codon usage of cDNA sequences of the original and optimized SCN1A and of SCN5A was analyzed by calculated the %MinMax score using an 18 base sliding window and the http://www.codons.org/ online server. % MinMax reports the usage frequency of a codon compared with the maximum, minimum and average possible usage for the given amino acid sequence.