Supplemental Methods

For the data shown in Supplemental Fig. 1, human Navβ4 was transiently transfected into stable HEK293 cells lines expressing Nav1.7-WT, Nav1.7-T1464I and Nav1.7-M1627K using the calcium phosphate precipitation technique. The calcium phosphate-DNA mixture was added to the cell culture medium and left for 15–20 hr, after which time the cells were washed with fresh medium. Sodium currents were recorded 48-72 hr after transfection.

Supplemental Figures and Legends

No peptide				
Channel	**I_{peak} (nA)[a]**	**Capacitance (pF)**	**Current density (pA/pF)**	***n***
Nav1.7	**2.93 ± 0.36**	**17.3 ± 0.7**	**169.6 ± 20.2**	12
Nav1.7-M1627K	**1.27 ± 0.30**[#]	**16.9 ± 0.7**	**70.2 ± 16.6**[#]	9
Nav1.7-T1464I	**1.28 ± 0.40**[#]	**18.7 ± 1.2**	**74.3 ± 24.5**[#]	8
Nav1.7-V1299F	**0.78 ± 0.18**[#]	**16.6 ± 0.7**	**42.3 ± 8.5**[#]	9
Nav1.7-I848T	**1.93 ± 0.64**	**17.9 ± 1.2**	**107.0 ± 38.4**	8
Nav1.7-L858H	**0.67 ± 0.09**[#]	**17.5 ± 0.8**	**38.2 ± 5.3**[#]	8
Navβ4 C-terminal peptide (100 μM)				
Channel	**I_{peak} (nA)[a]**	**Capacitance (pF)**	**Current density (pA/pF)**	***n***
Nav1.7	**2.10 ± 0.26**	**15.3 ± 0.5**[†]	**134.9 ± 14.1**	14
Nav1.7-M1627K	**0.71 ± 0.08**[#]	**16.8 ± 0.7**	**42.5 ± 5.2**[#]	11
Nav1.7-T1464I	**1.23 ± 0.33**	**16.6 ± 1.1**	**76.9 ± 18.8***	11
Nav1.7-V1299F	**0.86 ± 0.27**[#]	**15.8 ± 1.2**	**66.1 ± 26.2***	11
Nav1.7-I848T	**2.02 ± 0.44**	**17.5 ± 1.9**	**109.9 ± 23.1**	10
Nav1.7-L858H	**0.87 ± 0.17**[#]	**20.4 ± 1.5**[#]	**44.5 ± 10.2**[#]	8

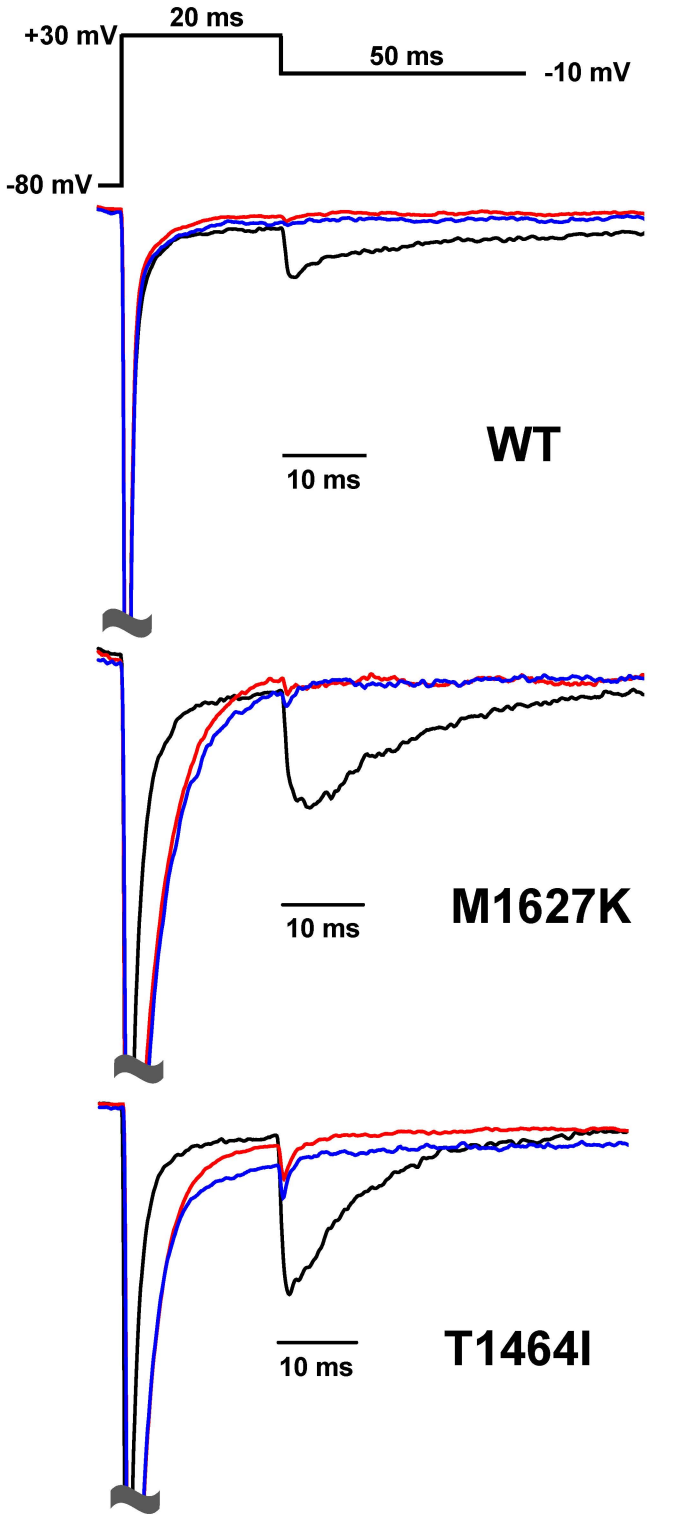
Supplemental Table 1. Peak current, capacitance and current density of WT, PEPD and IEM Nav1.7 channels. [a]The peak current was measured with a depolarizing pulse to +20 mV from a holding potential of -80 mV. *$p < 0.05$, [#]$p < 0.01$ from Nav1.7. [†]$p < 0.05$ from no peptide by unpaired student's t-test.

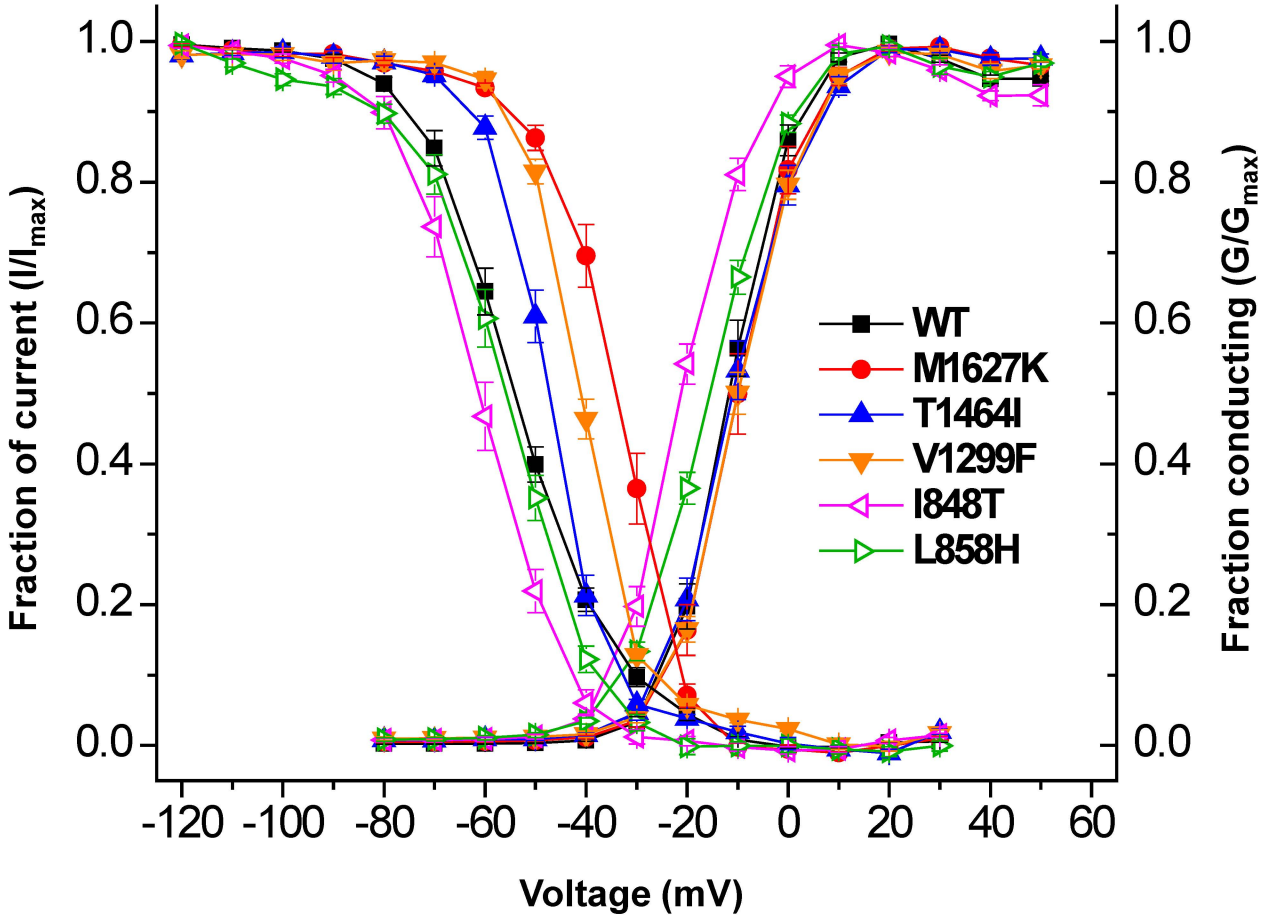
Supplemental Figure 1. Co-expression with full-length Navβ4 subunit does not induce resurgent currents with wild-type Nav1.7 or PEPD mutants. Voltage protocol and normalized resurgent current traces recorded from representative conditions, as labeled. Traces were normalized to the peak transient current evoked at +30 mV. The black traces (Nav1.7 + Navβ4 peptide) were taken from Figure 3.

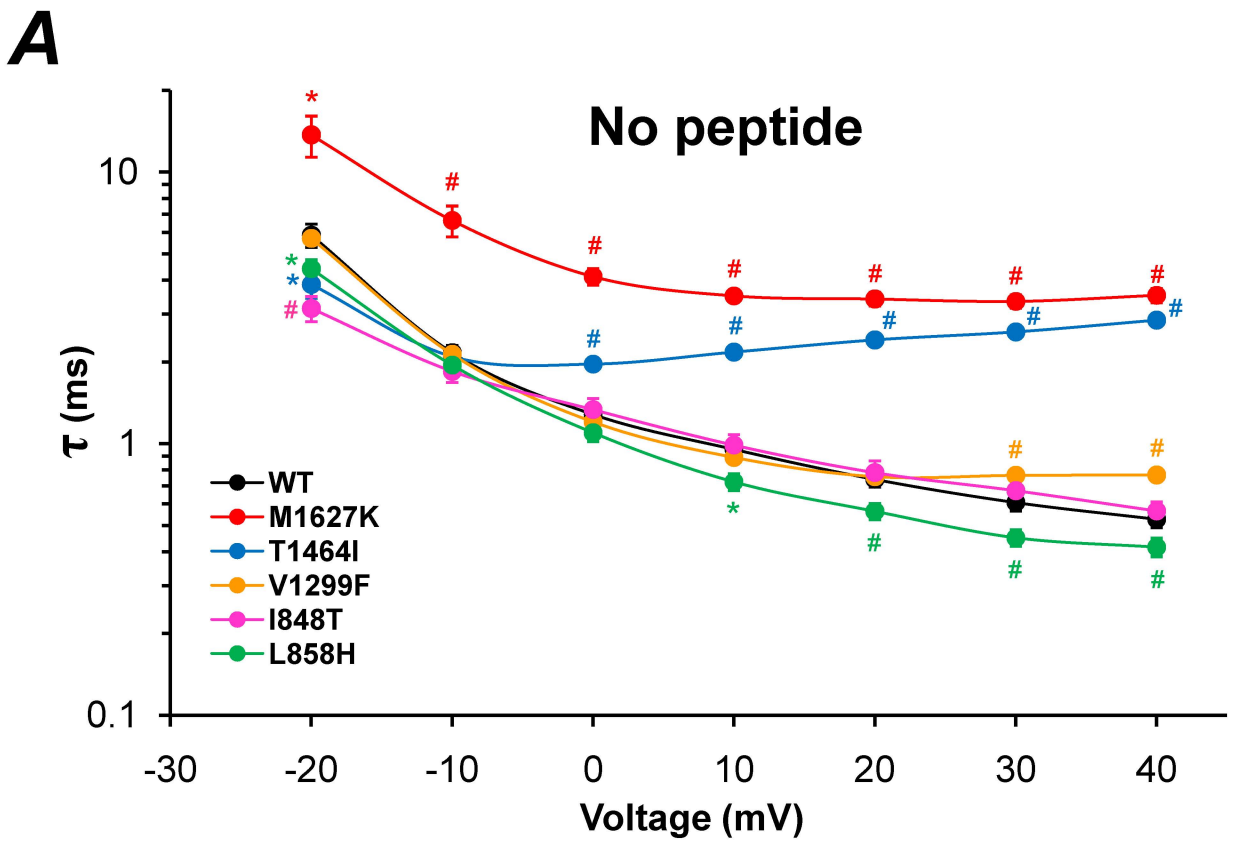
Supplemental Figure 2. Steady-state fast inactivation and activation curves for WT, PEPD and IEM Nav1.7 channels: Steady-state inactivation profiles were determined using a stepwise conditioning pulse from −120 mV to +30 mV (in 10 mV increments) for 200 ms and followed by a test pulse (+15 mV, 20 ms) and fit with a Boltzmann function. Voltage-dependent conductance properties were derived from I/V data and fitted with a Boltzmann function. The data presented are in the absence of the Navβ4 peptide.

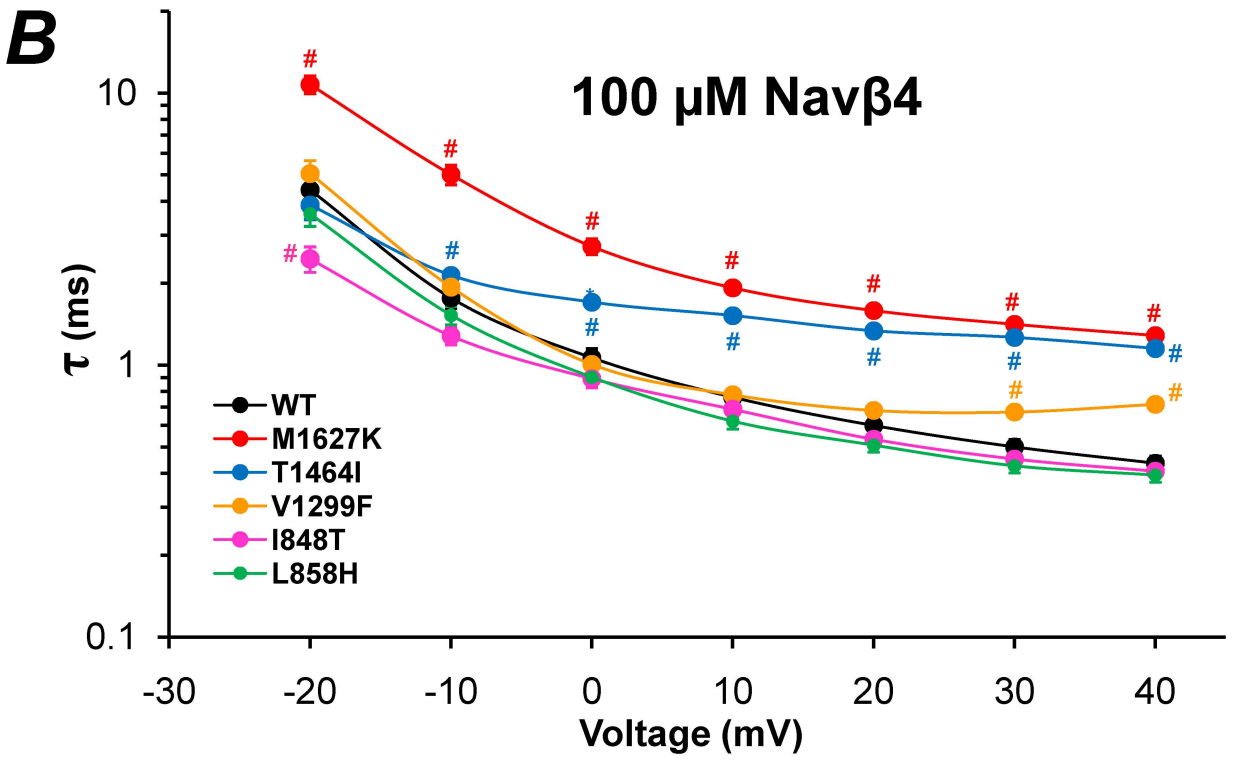
Supplemental Figure 3. PEPD mutations slow the rate of fast inactivation across multiple potentials. Line graph displaying the average decay time constants (τ) in the absence (*A*) and presence (B) of Navβ4 peptide in the recording pipette across test potentials ranging from -20 to +40 mV (n = 8-14, $^{*}p < 0.05$, $^{\#}p < 0.01$ by unpaired student's t-test different from WT).

Supplemental Figure 4. Inclusion of the Navβ4 peptide accelerates the transition to the non-conducting state. Decay time constants (τ) with and without the Navβ4 peptide for each individual construct as labeled at each corresponding test potential. Different scales were used for each of the plots to emphasize the differences between the decay time constants with and without the peptide (n = 8-14, $^{*}p < 0.05$, $^{\#}p < 0.01$ by unpaired student's t-test different from Navβ4).


+30 mV
20 ms
50 ms
-10 mV
-80 mV
10 ms
WT
10 ms
M1627K
10 ms
T1464I
Nav1.7
Nav1.7 + Navβ4 full-length protein
Nav1.7 + Navβ4 peptide


Fraction of current (I/I$_{max}$)
Fraction conducting (G/G$_{max}$)
Voltage (mV)
1.0
0.8
0.6
0.4
0.2
0.0
-120
-100
-80
-60
-40
-20
0
20
40
60
WT
M1627K
T1464I
V1299F
I848T
L858H


A
No peptide
τ (ms)
10
1
0.1
-30
-20
-10
0
10
20
30
40
Voltage (mV)
WT
M1627K
T1464I
V1299F
I848T
L858H


B
100 μM Navβ4
τ (ms)
10
1
0.1
-30
-20
-10
0
10
20
30
40
Voltage (mV)
WT
M1627K
T1464I
V1299F
I848T
L858H

Supplemental Figure 4